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The Use of Geographic Information Systems and Ecological Niche Modeling to Map Transmission Risk for Visceral Leishmaniasis in Bahia, Brazil

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THE USE OF GEOGRAPHIC INFORMATION SYSTEMS AND ECOLOGICAL NICHE MODELING TO MAP TRANSMISSION RISK FOR VISCERAL LEISHMANIASIS IN BAHIA, BRAZIL

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Abstract

Leishmaniasis is a public health problem in Brazil, and despite the control programs in place, Bahia remains one of the states with the highest incidence rates of cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). This study proposed to develop and evaluate the applicability of ecological niche models (ENM) for leishmaniasis, to determine the influence of environmental and socioeconomic factors on the incidence of these diseases at three geographic scales: national, statewide and community. Water availability, garbage collection, precipitation and temperature were the most important variables in predicting areas suitable for VL and CL in the country. The extensive geography of Brazil and the coarse scale of the data used to evaluate both socioeconomic and environmental variables revealed the need of a more refined scale to define the role of these factors in risk area identification. At the statewide scale, the models were developed for Bahia state and data on vector occurrence was added to the analysis. Three environment structural indices were evaluated in addition to the environmental variables explored in the national model. Water content of vegetation was a very strong predictor of CL and VL incidence followed by NDVI. The sand fly species found in Bahia were sensitive to variations in temperature and rainfall related variables. The occurrence of *Lutzomyia longipalpis,* the vector of VL in the state, was most influenced by precipitation and vegetation. The district of Monte Gordo, in Bahia, was selected for development of a community level ENM using high resolution WorldView-2 imagery. CDC light traps were used to collect sand flies for a period of three months. Sand flies were tested by polymerase chain reaction (PCR) to determine host feeding preferences and natural infection by *Leishmania spp.* The sand flies preferably fed on chickens and humans. No natural *Leishmania spp*. infections were detected. NDVI was the most influencing factor in the ENM model (99.4% contribution). Implementation of a multi-scale geospatial surveillance and risk modeling capability to monitor disease incidence and their vectors, with the addition of molecular analysis, into the actions of the control program can help reduce the impact of endemic leishmaniasis in Bahia..

Chapter 1: Introduction

Leishmaniasis is caused by flagellated protozoans of genus *Leishmania* Ross 1903, which are transmitted by phlebotomine sand flies of the genus *Lutzomyia* (American Continent) (Young and Duncan, 1994). These diseases are amongst the six most important neglected tropical diseases and are considered a serious public health issue. Disease is manifested in different clinical forms, cutaneous, muco-cutaneous and visceral (Peters and Killick-Kendrick, 1987).

The life cycle of these parasites are associated with a vertebrate host, in which the amastigote form of the parasite lives in mononuclear phagocytic cells, and an arthropod host (phlebotomine sand flies), in which the promastigote form of the parasite develops and multiplies within the host digestive tract (Grimaldi and Tesh, 1993). The main reservoirs for leishmania species are wild mammals such as foxes, opossums, and rodents. Domestic animals such as dogs and horses are also associated with maintenance of infection source, thus maintaining the life cycle of this parasite (Brasil, 2007; Lainson and Shaw, 1998; Rangel, 1995).

In Brazil, leishmaniasis is reported in all states, with an increasing incidence in recent years, especially in urban areas. About 93% of the cases of the visceral form of leishmaniasis (VL) reported in Latin America occur in Brazil. The Northeast region of the country reports the majority of the cases including Bahia state. Changes in the transmission patterns of VL due to man-made environmental changes and urbanization of cities has been observed. This endemic disease was previously associated to rural environments but is now associated with the periphery and urban areas of large cities (Brasil, 2006; Brasil, 2011; PAHO, 2013; Rangel, 1995).

VL was previously restricted to the central region of Bahia but has expanded to the surrounding areas, which were previously free of disease. Approximately 52% of the municipalities in Bahia have reported autochthonous cases of VL (Bahia, 2011). Amongst those municipalities, we selected the district of Monte Gordo for the current study due to its endemic characteristic and intense transmission of VL foci for a refined scale ecological niche modeling (ENM) of VL vector in the area. The study design was to develop ENM for leishmaniasis, mainly focusing on the visceral form and the vector in different scales. First, models for cutaneous and visceral leishmaniasis were developed for the entire country (Brazil). Secondly, the models were refined to a state scale where both forms of leishmaniasis and the occurrence of sand flies were modeled in Bahia. Lastly, a community scale model was developed for Monte Gordo, based on captured sand flies in the area; feeding preferences and natural infection by leishmania in the collected sand flies was also analyzed at the community scale.

Environmental changes and ecological disturbances can exert an influence in the establishment and dispersion of diseases by altering the ecological balance and context in which vectors and parasites breed, develop and transmit disease (Patz et al., 2000). The identification of ecological niches in the study of vector-borne diseases makes it possible to identify components of infection risk.

Ecological niche modeling (ENM) can be used to determine species distribution by estimating the relationship between species records at sites and the environmental and/or spatial characteristics of those sites. Knowledge of the mechanisms that influence the incidence of vector-borne diseases related to changes in the environment can contribute to the planning of control strategies including correct application of chemical control (Barata et al., 2005; Franklin et al., 2009; Peterson, 2006; Quintana et al., 2012; Salomón et al., 2011; Walsh et al., 1993).

Just as important as identifying the environment in which vectors thrive is the identification of feeding preferences of vectors transmitting agents of diseases and their natural infection rates. *Lutzomyia longipalpis* is a very versatile species and can feed on humans, dogs,

birds, and other vertebrates. This species has an opportunistic feeding behavior, thus it is important to generate knowledge about feeding preferences so specific control actions can be taken in specific risk areas (Missawa et al., 2008). Identification of sand flies naturally infected with leishmania provides understanding of vector-borne pathogen transmission cycles, and determination of the occurrence of naturally infected sand flies is an important step towards verification of specific vector species involved in disease transmission (Carvalho et al., 2010; Killick-Kendrick & Ward 1981; Rosa et al., 2012).

The objectives of the present study were to identify environmental and socioeconomic factors associated with the occurrence of CL and VL in Brazil in the years 2005 to 2009, using RS, GIS and ENM techniques to predict the risk of disease at the municipality level (Chapter 3); to use products from NASA Earth Observing Systems (EOS) to calculate three different vegetation indexes, extract precipitation and elevation data, and then evaluate their applicability in the study of leishmaniasis in Bahia state, Brazil (Chapter 4); to analyze the spatial distribution for VL in humans, sand fly species, more specifically *Lu. longipalpis* in Bahia state, identifying environmental factors that can influence the distribution of vector species and their correlation with disease incidence (Chapter 5); to detect feeding preferences and natural *Leishmania* infection in sand flies collected in Monte Gordo, Bahia State, and identify priority areas for intervention based on a community level ENM of sand flies based on high resolution WorldView2 imagery (Chapter 6).

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Chapter 2: Literature Review

This chapter describes credible research experiences that guide the object of this dissertation evidencing studies that represent interfaces between the utilization of geographic information systems, ecological niche modeling and leishmaniasis.

2.1. Leishmaniasis

Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) are diseases of great clinical and epidemiological importance that mainly affect poor people in developing countries and have been listed by the World Health Organization as among the world's most neglected tropical diseases in urgent need of new research to develop more effective control measures. CL and VL are parasitic diseases caused by an obligated intracellular protozoan belonging to the order Kinetoplastida, family Tripanosomatidae, genus *Leishmania* (Bryceson, 1996). The generic denomination *Leishmania* was given by Ronald Ross after two parasitologists, W. B. Leishman and C. Donovan, whom in 1903, separately but simutaneously demonstrated parasites in stained smears from the spleen of patients suffering from malaria-like illness. Nicolle em 1908 reported that mammals, including canines could act as reservoirs hosts for *Leishmania* parasites (Humber, 2005).

Leishmaniasis is transmitted by the bite of infected female sand flies belonging to either *Phlebotomus spp*. (Old World) or *Lutzomyia spp*. (New World) (Order: Díptera; Family: Psychodidae; Subfamily: Phlebotominae). Canines, rodents, and opossums, are among the mammalian host species that are proven or suspected reservoir hosts (Ashford, 1996; Ready, 2013). Leishmaniasis is manifested as three main clinical syndromes: cutaneous leishmaniasis, muco-cutaneous leishmaniasis, or visceral leishmaniasis (Murray et al., 2005; WHO, 2010). Visceral leishmaniasis (VL), the most severe form of the disease which is fatal if left untreated, is a systemic disease caused by species of the *Leishmania donovani* complex in East Africa

and the Indian subcontinent and by *Leishmania infantum* in Europe, North Africa and Latin America (Figure 1) (Lukes et al., 2007; Mauricio et al., 2000).

Infection in people is caused by more than 20 species of *Leishmania*, which are transmitted by about 30 species of phlebotomine sand flies. Particular species of the parasite are transmitted by particular sand flies. The species involved in the infection determines how the disease will evolve. Thus, it is essential to know the species involved in each focus, as this knowledge may impact epidemiological understanding, control measures and treatment (WHO, 2010).

The geographical distribution of leishmaniasis is restricted to tropical and temperate regions. The leishmaniases are considered to be endemic in 88 countries on four continents. About 90% percent of cases with cutaneous forms of leishmaniasis occur in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria. Regarding the visceral form of the disease, the majority of the cases are found in Bangladesh, Brazil, India, Nepal and Sudan (Figure 2). Over 90% of potentially fatal infections of VL occur in just six countries: Brazil, Ethiopia, Sudan, South Sudan, India, and Bangladesh. The World health Organization (2015) estimates an occurrence of 1.3 million new cases of this endemic disease with 20,000 to 30,000 deaths annually. In several endemic countries, a dramatic increase in the number of leishmaniasis cases has occurred during the last decade. Prevention and control interventions are slowed by the diversity of the structure of leishmaniasis foci, with many different animals acting as reservoir hosts and the existence of a variety of sand fly vector species (Dedet, 2008). The factors driving the transmission of leishmaniasis are different between the Old and New World. Pigott et al., (2014) reported that built-up areas were more likely to be at risk factor in the Old World, while temperature and rainfall were more important factors affecting risk in the New World.

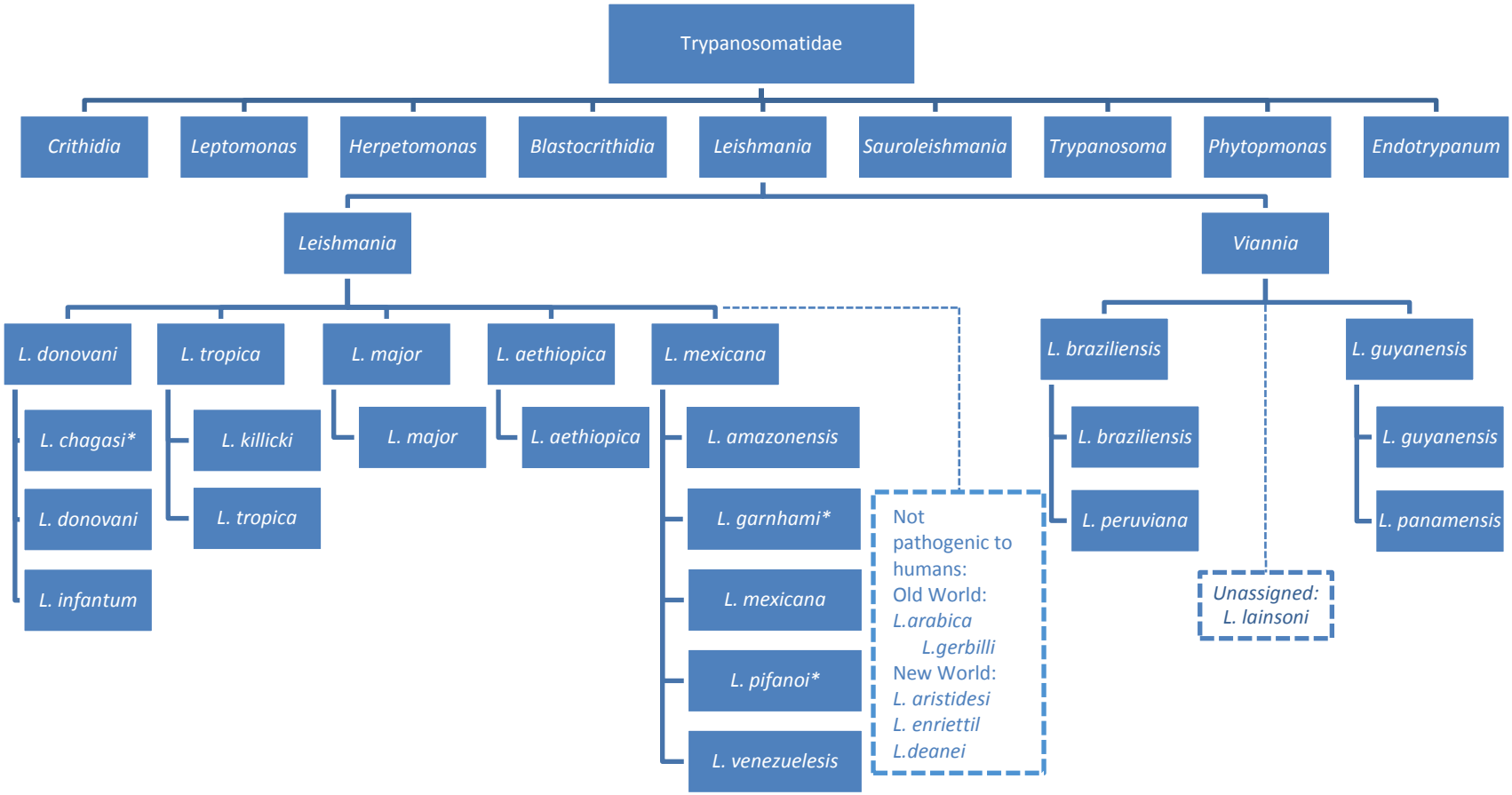

Figure 2.1. Taxonomy of Leishmania - *Species status is under discussion. L. chagasi in the New World is the same as L. infantum. Sauroleishmania has only been reported to infect lizards and reptiles. (Adapted from the World Health Organization technical report series n. 949 (2010).

Leishmaniasis, like many other NTDs, occurs in a focal distribution and in remote locations, making extrapolation from official data sources difficult. Previous estimates of the leishmaniases' global burden have been complicated by poor knowledge of the global distribution of these diseases. While in many countries data on the presence or absence of leishmaniases is available, in other areas, these assessments reveal significant uncertainty in assessing disease presence or absence using currently available evidence (Bern et al., 2008; Pigott et al., 2014; Reithinger, 2008). It is in such areas that surveillance efforts need attention so knowledge of the global distribution of leishmaniasis can improve.

Despite the lack of certainty about the global burden, the fact remains that leishmaniasis is increasing and the prospects for control are highly dependent on research progress to obtain better tools and more cost-effective strategies for case management and vector control (Desjeux, 2004). Though leishmaniasis represents a significant public health burden, the understanding of its global distribution remains vague, reliant upon expert opinion and limited to poor spatial resolution (Pigott et al., 2014).

2.1.1. Visceral Leishmaniasis

Visceral leishmaiasis, also known as Dum-dum fever, Sikari disease, Burdwan fever, Shahib's disease, tropical splenomegaly, kala azar or American visceral leishmaniasis, is caused by several subspecies of *Leishmania* in the tropical and subtropical regions and infection can be sporadic, endemic or sometimes epidemic. The most commonly used term is Kala azar, which in Hindi means black sickness or black fever. The terms originally referred to Indian VL due to its characteristic symptoms, blackening or darkening of the skin of the hands, feet, face and the abdomen (Lainson and Shaw, 1987).

The WHO (2015), estimates that 200,000 to 400,000 new cases of VL occur each year with approximately 59,000 deaths per year. Most of the cases of VL occur in the American

continent, with the majority of cases reported in Brazil (Grimaldi et al., 1989; WHO, 2010). In Brazil, VL represents a serious public health problem due to its scale and geographical extent.

This disease is considered endemic in Brazil and was previously described in rural zones with semi-arid climates. However, reduction of the natural ecologic space occupied by the parasite and vector adaptation to different habitats are the reason behind an increase in the incidence of disease as well as its shift from rural settings to urban centers of larger cities and occurrence in areas previously described as free of VL (Aguiar and Medeiros, 2003; Arias et al., 1996; Barboza et al, 2006; Deane and Deane, 1955; SINAN/SVS/MS 2010). Thus, environmental factors such as climate and vegetation no longer represent a barrier to the expansion of this disease (Deane and Deane, 1962; Rebêlo, 1999; Werneck, 2008).

Currently, VL is reported in 19 out of 27 states in Brazil, occurring in all five geographic regions. Between 1990 and 2013 a total of 74,980 VL cases were reported in Brazil, the Northeast region being the region with the highest number of cases reported, contributing with 67.15% of the records (SINAN/SVS/MS, 2014). Bahia state is one of three states in this region that most contribute to the reports of VL. The percentage of cases reported in the same period for Bahia corresponds to almost 28% and the incidence rate observed in 2012 was 2.0 cases/100,000 inhabitants with about 52% of the municipalities reporting autochthonous transmission (Bahia, 2012; Mutebi et al., 1999; SINAN/SVS/MS, 2013).

2.1.2. Epidemiology and Transmission

The parasites that cause VL infect the spleen, liver, bone marrow and lymphnodes of affected mammals. A granulomatous cell-mediated immune response occurs and this will result in either subclinical disease with self-cure or in the clinical syndrome of VL. It can take a few days or weeks to several years for symptoms to develop, but the incubation period usually ranges from two to six months (Garcia, 2001).

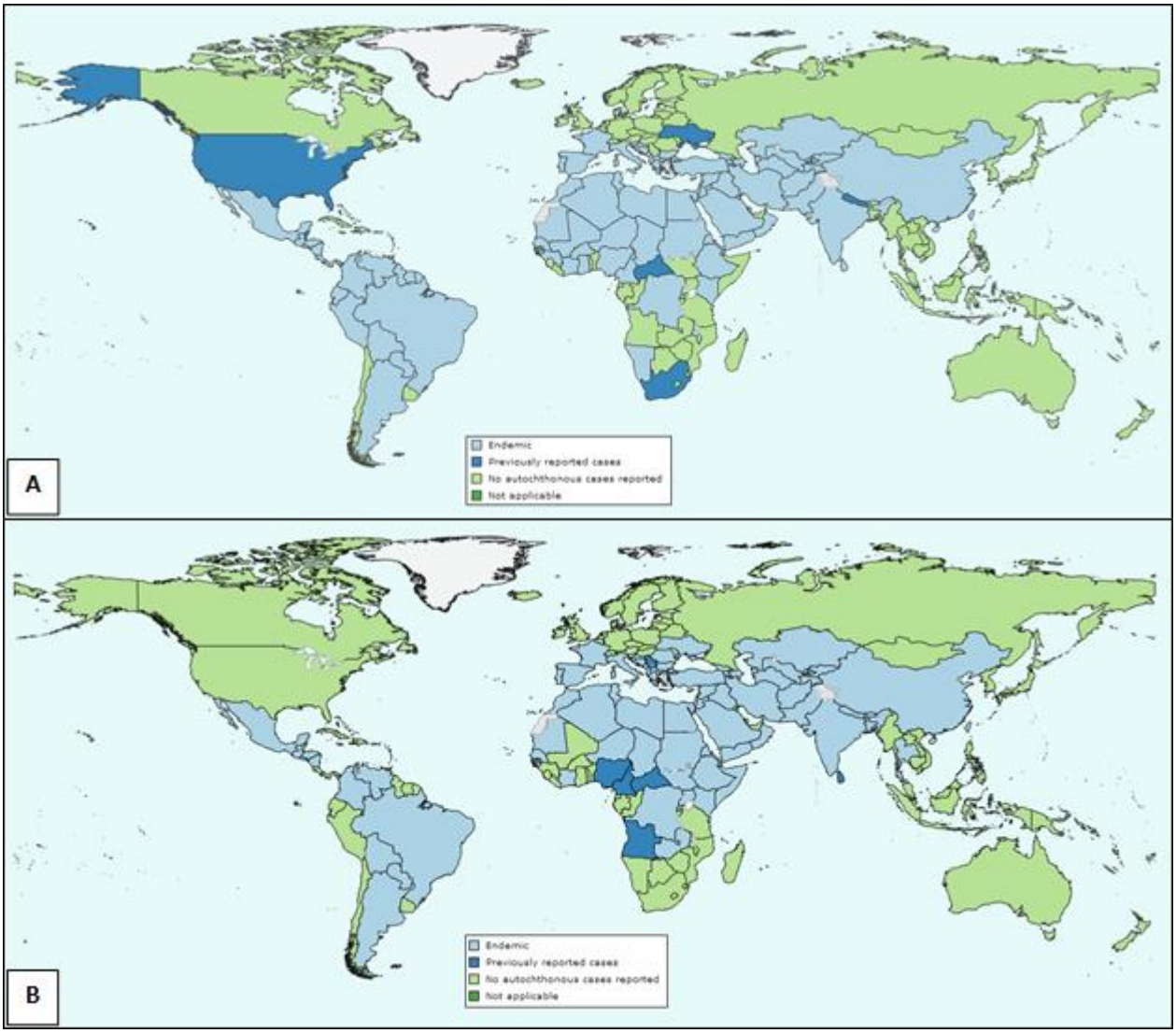

Figure 2.2. Leishmaniasis: Status of Endemicity - Cutaneous (A) and Visceral (B) leishmaniasis by country. (Adapted from WHO, interactive graphs, 2013).

Infected people usually develop fever, chills, anorexia, malaise, weight loss, diahrrea, and enlargement of liver and spleen (Figure 3). Mortality rate can reach up to 90% if the disease is left untreated (Desjeux, 1996; Boelaert et al., 2000). The disease is also associated with age, young children are the most affected, especially when their health is compromised by nutritional deficiencies or immunosupression, which is often observed in VL-HIV co-infection (Gramiccia and Gradoni, 2005; Thompson et al., 2002).

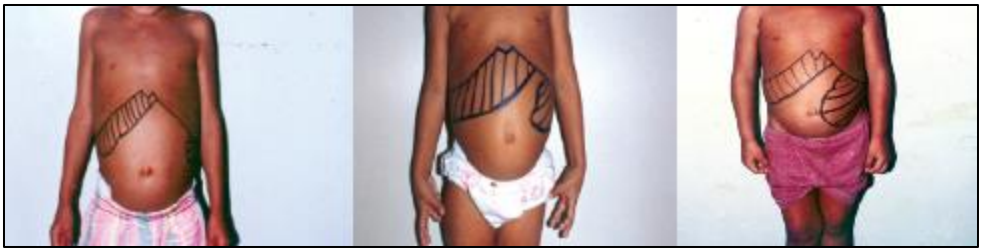

Figure 2.3. Classical Symptoms of VL - Infected children displaying hepatosplenomegaly (Brasil, 2014).

Parasites of the genus *Leishmania* are obligate, intracellular parasites that infest more than one host species, requiring at least two host species to complete the life cycle. The life cycle of *Leishmania* is relatively simple, involving a mammalian host and a vector, which presents two distinct morphological stages: the intracellular amastigote, parasitizing the cells of the mononuclear phagocyte system of the mammalian host; and the flagellated promastigote, a motile infective form of the parasite that develops in the intestinal tract of the arthropode vector. The parasite's cycle in the vector is completed in about 72 hours (Dedet, 2008; Ready, 2014). Dissemination of the parasites occurr when a female sand fly ingest amastigote forms of the parasite while blood feeding on an infected mammal. The amastigotes are then transformed to promastigotes when they migrate from the sand fly intestines to the proboscis, transform into infectious metacyclic promastigotes and are then inoculated into the skin of a new host during the vector's next blood meal (Figure 4) (Boelaert et al., 2000; Esch and Petersen, 2013; Sacks and Kamhawi, 2001).

Humans can carry some species of *Leishmania* asymptomatically for long periods, without becoming ill. The reported incubation period for VL is usually from 10 days to 18 months with inflammatory reactions within the viscera becoming apparent in two to six months after infection, but VL symptoms can take years to appear. The disease is progressive and without treatment may lead to death in 75-95% of the cases. Death usually occurs within two years but spontaneous cure can occur (Barret and Croft, 2012; John and Petri, 2006).

Based on its transmission, there are two epidemiological characterizations that vary in different geographic regions: Anthroponotic transmission (human←sand fly→human), where humans are the main reservoir and source of infection for the vector, is mainly restricted to some countries in the Asian and African continent; and zoonotic transmission (human↔sand fly↔reservoir), where an animal reservoir host is involved in the transmission cycle and which occurs is semi-arid regions in Latin America and Mediterranean regions with the domestic dog serving as the mair reservoir (Ashford, 2000; Ready, 2014; Sherlock, 1996). Transmission however, is dependent on ecology, therefore, human infection vary in time and space. Introduction of humans into a sylvatic cycle results in exposure to the vector, thus leading to risk of infection.

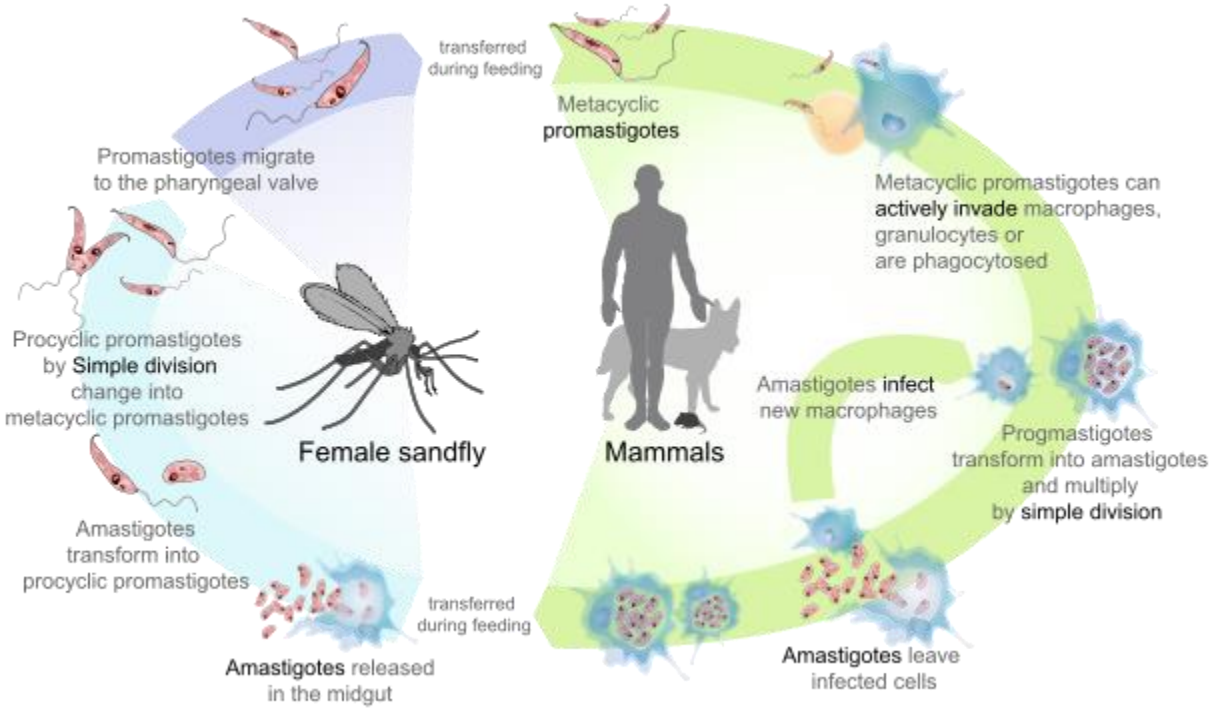

Figure 2.4. Life Cycle of the Parasites from the Genus Leishmania - By Lady of Hats Mariana Ruiz Villarreal (http://commons.wikimedia.org/wiki/File%3ALeishmaniasis_life_cycle_diagram_en.svg)

The domestic dog is the principal animal reservoir of VL and dog's constant proximity to human beings in areas where VL is endemic contributes to the dispersion of this disease during migration movements (Arias *et al.*, 1996; Moreira-Jr et al., 2003; Oliveira et al., 2001). Wild canids such as foxes have also been reported as natural reservoirs and are responsible for the disease in sylvatic cycle (Silva et al., 2001). Infection by *Leishmania spp*. have been occasionally observed in other domestic species such as cats (Ayllon et al., 2008; Martin-Sanchez et al. 2007; Sherlock, 1996; Silva et al. 2008), horses (Fernandez-Bellon et al. 2006), cattle (Bern et al., 2010), sheep (Anjili et al., 1998), pigs (Brasil et al., 1987; Moraes-Silva et al. 2006) as well as in wild animals or captive wild species, including the opossum (*Didelphis albiventris* and *Didelphis marsupialis*) (Cabrera et al., 2003; Santiago et al., 2007), crab-eating fox (*Cerdocyon thous*), hoary fox (*Dusicyon vetulus*), maned wolf (*Chrysocyon brachyurus*) (Luppi et al., 2008), among others.

Animals are often infected asymptomatically with *Leishmania* spp. The reported incubation period for *L. infantum* in dogs varies from three months to seven years. In some dogs, severe clinical signs occur soon after the animal becomes infected. Other dogs remain asymptomatically infected, in some cases for a lifetime but these animals can become ill at any time and most importantly, continue to be a reservoir for the parasite, thus providing continuity to the parasite's life cycle and placing humans at risk of infection.

2.1.3. Vector

Under natural conditions, the sand fly ensures the survival of the parasite and the transmission of the disease (Guizani et al., 2011). The main vectors associated with transmission of VL are insects that belong to the order Díptera, family Psychodidae, subfamily Phlebotominae. Only the female sand flies feed on blood to acquire amino acids and proteins necessary for laying eggs (Ready, 1979). About 900 sand fly species have been identified worldwide. In the American continent 60 species are recognized as vectors or potential vectors

(Peters and Killick-Kendrick, 1987). In Brazil, there are two confirmed species involved in the transmission of VL, *Lutzomyia longipalpis* and *Lutzomyia cruzi*, the latter only occurring in the central west region of the country, Mato Grosso do Sul state (Brasil, 2014) and in Pernambuco state, *Lutzomyia migonei* is being considered a possible vector of VL as well (Carvalho et al., 2010).

Sand flies are small, usually very hairy, with colors ranging from white to black (Figure 4). When at rest, they characteristically hold their wings at an angle above the abdomen and when approaching a host for feeding they typically hop around before settling down to bite (Killick-Kendrick, 1999). Identification of these insects is based on the morphology of the reproductive organs of males and females.

Molecular analysis is available but limited to species of entomological importance (Guizani et al., 2011). Sand flies are holometabolous insects, in which development goes from the egg through four larvae stages, pupae and adult. The duration of each stage varies according to species, climate and food source. Sand fly larvae are terrestrial, very active, and move considerably fast in search of food. Sand fly larvae feed on organic matter near the site of oviposition, however, before molting to a different stage, the larvae cease feeding and some species may travel short distances to drier locations (Mascari et al., 2011; Sherlock, 2003). Therefore, natural habitats of sand flies are notoriously difficult to find. Natural habitats of immature stages have been identified only for few species and include areas rich in decomposing organic matter, such as livestock housing, soil and leaf litter or forests floor (Aguiar and Medeiros, 2004; Deane and Deane, 1957). Environments that can provide protection from sudden environmental changes, with high humidity, low luminosity and good air flow are suitable for larvae development (Aguiar and Vilela, 1987).

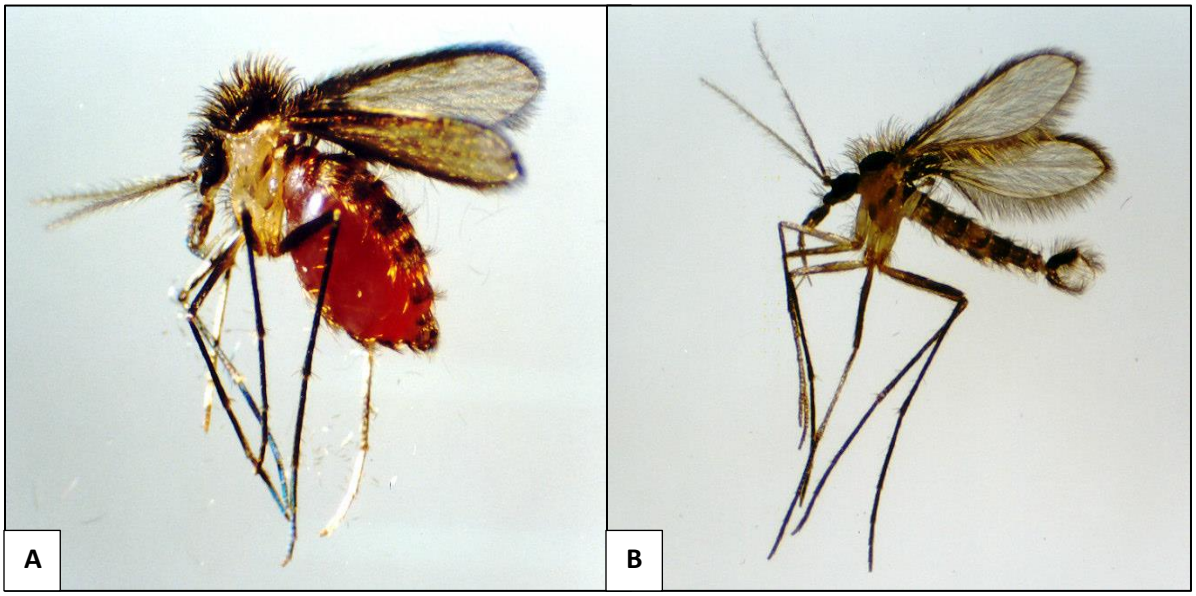

Figure 2.5. Sand Flies - Engorged Female (A) and Male (B) Sand Flies (Vale, 2008).

Because these insects are weak fliers, activity occurs when it is humid, and there is no wind or rain. They are usually most active at dawn, dusk and during the night, but they can bite if disturbed in their hiding places (animal burrows, holes in trees, caves, houses and other relatively cool, humid locations) during the day and in conditions of low luminosity. They are attracted to light and may enter buildings at night (Aguiar and Medeiros, 2003; Brazil and Brazil, 2003; Lewis, 1965; Killick-Kendrick, 1999).

In general, sand flies species are divided in three categories based on their habitats: sylvatic – for the species that inhabit forests or even non-forest environments, but are only accidently associated with humans and domestic animals; peridomestic – species that inhabit outside of human and animal housing, only seeking them for blood feeding; domestic – species that are associated with humans and domestic animals, living inside their houses or in proximity to them (Barreto, 1943).

2.1.3.1. Feeding Habits

As previously mentioned, sand fly larvae feed on organic matter present on soil. Like many other insects from the order Díptera, both male and female need carbohydrate supplements as their energy source for development, flying and mating. In nature, the main sources of carbohydrates are plant sap, nectar, honeydew of aphids and fruits (Alexander and Usma, 1994; Brazil and Brazil, 2003; Cameron et al., 1995). Only adult females are hematophagous, and need blood for oviposition. The ingestion of blood is associated with ovaries development and the number of eggs produced is directly proportional to the amount of blood ingested (Forattini, 1973; Lehane, 1991). The blood meal can take place between 24 to 48 hours after emergence and feeding time on the warm blood hosts can last from one to five minutes. The quantity of blood ingested varies according to the species and individual insect but it is generally the same as the insect's weight (Chaniotis, 1967). In most species, oviposition is preceded by a blood meal, but some species such as *Lutzomyia longipalpis* may blood feed a second time before laying eggs. The need for a second blood meal might be related to maintaining a hydric balance that can be affected by climate features and interfere with egg laying capacity (Brazil and Brazil, 2003; El-Naien et al., 1992). Such behavior is very important in the epidemiology of VL, since frequent blood meals increase the contact between vector and hosts, thus increasing the transmission ability of parasites by the sand fly.

Many sand fly species are opportunistic human-biters, but none are exclusively anthropophilic (Alexander, 2000). Host-feeding preferences can vary according to seasonal and environmental trends, region, host availability and abundance and factors intrinsic to the insect (Molaei et al., 2008; Turell et al., 2005). An example of a very versatile species is *Lutzomyia longipalpis* that is capable of feeding on man, canines, avian, and other animals in the same biotope with similar avidity. The attraction of sand flies to hosts involves amongst other stimuli, temperature and body odors (Brazil and Brazil, 2003). Identification of food source from hematophagous insects provide useful information for control program interventions.

2.1.3.2. *Lutzomyia longipalpis*

Lutzomyia longipalpis is the best studied sand fly in the American hemisphere because it is the principal vector of VL. Following the first description of the sand fly *Lutzomyia longipalpis* (Lutz and Neiva, 1912), in an indeterminate locality in Brazil, interest in this insect remained largely entomological until the mid-1930s. After the first records of VL in Brazil, in 1934, this species of sand fly was incriminated as the principal vector (Lainson and Rangel, 2005).

Lutzomyia longipalpis is geographically widespread and locally abundant, especially in locations where domestic animals are kept. This species has been found in a variety of ecosystems in Brazil ranging from rocky and dry environments to humid areas such as the Amazon basin (Barros et al., 2000; Galati et al., 2003; Lainson et al., 1990; Ribeiro et al., 2002). Despite its geographical distribution, one characteristic of this species is the capacity of adaptation to peridomestic environment, particularly in rural areas or peripheral areas of urban centers in any period of the year (Laison, 1989). Changes in its behavior as well as frequent exposure to insecticides can lead to insect population resistance to chemicals and this could be the reason for the population increase of this insect in urban areas. The opportunistic behavior expressed by this vector in relation to its food source, might have favored its vector competence.

Lutzomyia longipalpis is naturally infected with *Leishmania infantum* after feeding on an infected host; experimental infections with *L. amazonensis* and *L. braziliensis* have also been shown (Sherlock et al., 1996; Silva, 1990).

In the north and northeast regions of Brazil, it has been observed that this species can be found in all months of the year with higher sand fly density during the wet season or the transition between wet and dry seasons (Rebelo et al., 2001; Sherlock and Guitton, 1969; Sherlock et al., 1996). Long drought periods observed during El-Niño years have also been cited as influencing sand fly density and transmission intensity of VL by this vector (Franke et al., 2002). In the domicile or peridomicile areas, *L. longipalpis* is usually found in proximity to

food sources (Neves, 2005). In laboratory conditions, *L. longipalpis* is easy to maintain, very prolific and resistant to changes in environmental conditions (Rangel et al., 1987).

2.1.3.3. Vector Control

The distribution of VL is shaped by the distribution and abundance of the vector, which is dependent on environmental factors that can affect feeding, growth and breeding. The use of insecticides is reported as the best vector control strategy in the fight against VL and is among the measures recommended by the WHO. Sand flies are very sensitive to a variety of insecticides including those used in mosquito abatement (Dye, 1996; Guizani et al., 2011). Control of the vector is mainly focused on the adult stages of the insect, since natural habitats of immature stages are difficult to find. The goal of vector control of adult stages of sand fly species is to avoid or minimize contact between the vector and hosts/reservoirs. Environmental management is also an option in controlling sand flies but it may not be an effective tool since elimination of breeding sites or immature stages are dependent on knowledge of natural habitats as well. An obstacle in relying on the use of a single control method however, is the development of resistance to chemicals (Alexander and Marole, 2003).

The control measures for adult stages of sand flies include the use of insecticides for residual spraying of dwellings and animal shelters, insecticide-treated nets and dog collars, and personal protection by use of insect repellents. The chemical components currently used include organochlorine insecticides such as dichlorodiphenyltrichloroethane (DDT) and β-hexachlorocyclohexane (BHC), propoxur, malathion, fenithrotion, and synthetic pyrethroids. Organochlorine insecticides have significantly reduced sand fly population density in VL outbreak areas, thus reducing disease incidence (Oliveira-Filho and Melo, 1994). However, the Brazilian Government doesn't allow the use of organochlorines in agriculture and vector control programs due to high toxicity and environmental contamination (D'Amanto et al., 2002). So other insecticides have been utilized, such as synthetic pyrethroids. This chemical has had a

major impact on sand fly control in Brazil, reducing the number of sand flies indoors after the use of deltamethrin, decreased populations of *L. longipalpis.* The combined use of deltamethrin in the peridomicile and removal of organic matter from the soil surface can significantly reduce sand fly populations (Barata et al., 2011; De Silans et al., 1998; Falcão et al., 1991; Marcondes and Nascimento, 1993; Teodoro et al., 2003).

The Ministry of Health in Brazil recommends that in areas of moderate and intense VL transmission application of insecticides should be performed in periods of increased vector density. The limitation of this measure is that the seasonality curve of vector population needs to be known. In situations where vector seasonality is unknown, the first treatment cycle is initiated at the end of the rainy season and 3 to 4 months after the first cycle (Brasil, 2014).

Domiciliary and peridomiciliary fumigation provide good results in the population control of vectors of leishmaniasis. However, for fumigation to be effective, it needs to be carried out continuously, during appropriate seasons and periodically (Alencar, 1983; Nery-Guimarães and Bustamente, 1654; Sherlock and Almeida, 1970). The evaluation of the use of insecticides to control the sand fly is of fundamental importance. It is necessary to check the impact, the persistence of the insecticide on treated surfaces and the effectiveness of the product in relation to vector mortality (Brasil, 2014).

Effective vector control, however, cannot rely on a single control method. An effective vector control program needs alternative measures appropriate to different local realities. The planning of integrated vector control takes into consideration the environmental conditions and population dynamics of the vector. Appropriate control methods aim to maintain vector populations at levels that do not cause harm to health. Therefore, integrated vector control, is a system that combines appropriate control techniques to reduce the population of a pest insect, in order to keep the population below the nuisance level.

Environment management combined with chemical control and personal protection can provide effective control for VL. The WHO (1982) defines environmental control as the planning,

organization, implementation and monitoring activities for modification and/or alteration of environmental factors, or their interactions with man, in order to reduce to a minimum the spread of vectors and reduce contact between the man, the vector and the disease agent. Environmental control includes either measures of environmental modification, manipulation or modifications on human behavior. It consists of any physical transformation of land, water or vegetation, addressed to prevent, eliminate or reduce habitats of vectors, without causing undue adverse effects on the quality of the urban environment. Strategies such as cleaning yards, land and public squares provides changes in the conditions of the environment that provide the breeding establishment of immature forms of the vector. Street cleaning, removal and disposal of organic solid waste, non-permanence of domestic animals indoors can certainly help to prevent or reduce vector proliferation, thus reducing disease incidence.

2.1.4. Diagnosis and Treatment

Despite advances made in the past several years in the large number of diagnostic tests available, none of them have 100% sensibility and specificity. Diagnosis and treatment of patients must be performed early and, whenever possible, parasitologic confirmation of disease should precede treatment. In situations where serological or parasitological diagnosis are not available, however, the initiation of treatment should not be delayed (Brasil, 2014).

Diagnosis in humans is routinely performed based on clinical and epidemiological parameters. However, a definitive diagnosis requires demonstration of the parasite by parasitological methods. Clinical diagnosis of VL should be suspected when the patient has fever and splenomegaly associated or not with hepatomegaly. However, diagnosis and treatment follow-up always represents a challenge to physicians working in endemic areas due to the difficulty in diagnosing the disease clinically during its early phases.

Examination of Giemsa-stained slides is still the most commonly used technique to visualize the parasite. Other conventional methods for parasitological diagnosis include in-vitro

culture of infected tissue or inoculation into experimental animals. Parasitological diagnosis relies on spleen, bone marrow or lymph node aspirates. Common serological tests include the immunofluorescent antibody test (IFAT), direct agglutination test (DAT), enzyme-linked immunosorbent assay (ELISA), fast agglutination-screening test (FAST), and a rapid immunochromatographic assay (K39 dipstick or strip-test). Other assays include gel diffusion, complement fixation, indirect hemagglutination and countercurrent electrophoresis (Desjeaux, 2004; Herwaldt, 1999; Ready, 2014). Molecular diagnosis, such as polymerase chain reaction is more sensitive than microscopic examination of the parasite and is often the primary test in reference centers. *Leishmania* species that cause VL can be identified by DNA sequence analysis of polymorphic metabolic enzymes, restriction fragment length polymorphism analysis of the heat-shock 70 gene and by microsatellite DNA analysis (Gouzelou et al., 2012; Mauricio et al., 2006; Montalvo et al., 2012).

Visceral leishmaniasis can usually be cured in immunocompetent individuals. The pillar treatment for leishmaniasis has been the administration of pentavalent antimonials since the 1940's. Pentavalent antimonials can be used where the parasites are sensitive to these drugs, but resistance is a major problem in some areas. Other drugs such as allopurinol, amphotericin B or liposomal amphotericin B, and miltefosine are also used for treatment of VL. Amphotericin B is an alternative first choice treatment where antimonials are not indicated (CFSPH, 2009). In Brazil, pentavalent derivatives are the first choice drug for treating VL, where care for leishmaniasis is provided for free. The government offers liposomal amphotericin B (AmBisome, Gilead), meglumine antimoniate (Glucantime, Aventis), pentamidine and conventional amphotericin B for the treatment of leishmaniasis and all patients have access to treatment in the public sector. Meglumine antimoniate and amphotericin B (lipid formulations and conventional) are included in the National Essential Drug List for VL. Glucantime (Aventis) and AmBisome (Gilead) are registered in Brazil. Amphotericin B (AmBisome and conventional) are available at private pharmacies.

The cure rate with antimonials for VL is 83%. Cure criteria is essentially clinical. Follow up of the treated patient must be done at 3, 6 and 12 months after treatment and at the last evaluation, if the patient remains stable, the patient is considered cured (Brasil, 2014; Maia-Elkhoury and Gomes, 2008).

2.1.5. Prevention and Control

The main goal of VL prevention is to avoid host infection and subsequent disease. Control programs are intended to interrupt, limit or eradicate the disease for which control is being applied. Due to the structure and dynamic diversity of leishmaniasis foci, standard control programs cannot be defined and control measures must take into consideration unique local needs. Considering the several difficulties in elaboration of control policies for leismaniasis, the most significant is probably the high complexity of the eco-epidemiological features of this disease. Thus, control strategies depend on the ecology and behavior of reservoir hosts and vectors (Dedet, 2008).

Notification of leishmaniasis is mandatory in Brazil. A national leishmaniasis control program has been in effect in the country since 1985. Strategies to control VL in Brazil focus on early diagnosis and appropriate treatment for human cases, supervision and monitoring of the canine population and the elimination of dogs that test seropositive or positive for the parasite. Strategies also entail entomological surveillance, environmental health and chemical control measures, using residual insecticide, and finally preventive measures targeting humans, the vector and dogs (Gontijo and Melo, 2004). Despite the control measures in place, it has not been possible to prevent the geographic expansion and increase in incidence and lethality rates of this disease (Dantas-Torres et al., 2006). Strategies for the control of leishmaniasis in Brazil have not changed over the past 60 years, which might explain why incidence has not decreased (Dantas-Torres and Brandão-Filho, 2006).

Treatment of human patients may be helpful in areas where anthroponotic transmission occurs. Decreasing the incidence of *Leishmania* species in dogs can help protect people from this organism, but there are currently doubts about the efficacy of culling seropositive canines in eliminating infection sources for the vector. Vaccines to protect against leishmaniasis are either in the experimental or evaluation phase (Miró et al., 2008). Control perspectives are dependent on research to obtain better alternatives and management strategies of cases and vector control (Desjeux et al., 2004). Preventative measures against sand flies include using insect repellents, covering exposed skin, and staying on higher floors of buildings in the evening or at night, as these insects are poor fliers. Fans can also be helpful, and insecticidal sprays can be used to kill the insects inside houses. Insecticide-treated bed nets decrease bites from these insects at night (Killick-Kendrick, 1990; Feliciangeli, 2004). Besides being neglected in some areas and the risk of disease establishment in new areas, it is a fact that constant surveillance by health personnel is indispensable, as is implementing health education measures in endemic areas (Marcondes and Rossi, 2013).

Since leishmaniasis mainly affects poor countries, research and development of new tools and drugs have been somewhat neglected due to the fact that such countries may lack the resource necessary to invest in research. There is a clear need for a more cost-effective case management and vector control practices, and more investment and attention is needed in field oriented research to improve disease management, use of existent tools and validation/creation of new tools. Despite the progress that has been made in understanding its epidemiology, control of this endemic disease remains unsatisfactory (Dedet, 2008; Desjeaux, 2004).

2.2. Geospatial Technologies

Techniques used in collecting, processing, analysis and storage of geographically referenced information are powerful tools for decision making. Such technologies represent ways of providing deeper knowledge about population health, through the use of geo-referenced

maps, which allow for observations of spatial distribution of these diseases and corresponding risk areas.

Geographic Information System (GIS) is a computer-assisted information management system of geo-referenced data. It integrates acquisition, storage, analysis and display of geographic related data. Among its many applications, GIS is a tool that can be used to assist in health research, education, planning monitoring and evaluation of health programs. The spatial modelling capabilities offered by GIS can help in understanding the spatial variation in disease incidence and its relationship to environmental factors. Maps are excellent means of communication, thus GIS can be used to prepare educational material for helping people to understand their environment (Loslier, 1994).

GIS provides a digital lens for exploring the dynamic connections between people, animals, their health and well-being, and changing physical and social environments. Many applications of GIS in public health include management of available health resources, prediction, simulation and management of epidemics and monitoring and control of diseases. Health professionals can map the occurrence of endemics and act directly in places where they occur, increasing their chances of success in controlling diseases. GIS also makes it possible to build maps of disease distribution or occurrence, to prioritize areas for specific control, thus improving resource allocation (Bavia, 1996; Bavia et al., 2001; Peterson et al. 2000).

GIS can have a profound impact on public health strategies involving surveillance, risk assessment, analysis, and the control and prevention of diseases. One of its main characteristics is the ability for inserting and integrating spatial information from cartography, census data, networks and terrain models, and satellite data into a single database. At the same time, GIS software offers mechanisms to combine information through algorithms for manipulation and analysis as well as ways of consulting, visualizing, retrieving and plotting of the geo-referenced database contents (FatorGIS, 2005).

Bavia et al., (2001) described GIS as a powerful analytical tool. It contemplates a broad and qualitative universe of options in assessments and simulations of different scenarios. GIS consists of a computerized data system capable of handling a lot of information related to a specific geographical area and proceed to the storage, analysis, manipulation, interaction of multiple variables and integrated or isolated exhibition of data while also enabling the simulation of systems capable of predicting events and respond to numerous research questions.

With the use of techniques within a GIS, it is possible to choose the areas to implement control measures against VL in a more effective way (Oliveira et al., 2001). But building a GIS focused on the epidemiological surveillance is no easy task, the geo-referenced data is not readily available in some cases. Cartographic information are still difficult to obtain despite the efforts of the Brazilian Ministry of Health and the Pan American Health Organization (PAHO) (Carvalho et al., 2000). Finally the methods of analysis, even the simplest models of interpolation are not yet at the user-friendly level in the departments of health. However, the experience being accumulated in the study and control of this endemic disease can contribute substantially to the development of spatial analysis tools focused on epidemiological surveillance (Camargo-Neves et al., 2001). Overall, GIS facilitates access to epidemiological data through visualization and can also be used for development of models and spatial statistic analysis (Bergquist et al., 2011).

Remote Sensing (RS) is a science in which the goal is to develop techniques for extraction of information about objects on the earth's surface, through detection and measurement of interaction between electromagnetic radiation and terrestrial materials (Meneses, 2004). As a tool for analysis and environmental planning, RS and geo-processing allow for the visualization of spatial components in a local, regional and global scale (Cendrero, 1989). Remotely-sensed products allied with GIS techniques make possible the development of maps depicting suitable environments for the development and propagation of vector-borne disease (Malone, 2005). These techniques are becoming as important as fieldwork. Earth-

observing satellites are now often exploited for the measurement of environmental variables of relevance to epidemiology and public health. A wide variety of vector-borne diseases have been investigated using RS products and/or GIS techniques (Bavia et al., 2011; Cardim et al., 2008; Carneiro et al., 2004; Malone et al., 1997; Nieto et al., 2006).

The use of such health maps allows for the appropriate choice of disease control, since different control options are optimal in different endemic settings. It permits better planning of control strategies to be used considering the population at risk, and promotes better spatial targeting of areas for intervention and optimal timing to apply such control measures. These efforts can be achieved at different scales depending on the level of interest at the regional, local, or community level (Tatem et al., 2004).

Remotely-sensed information also provides useful observation on climatic, ecological and even anthropogenic factors related to transmission patterns of many diseases. Satellite technology assists both control measures and epidemiological research (Bergquist, 2011).

2.3. Ecological Niche Models (ENMs)

The relationship between diseases and their different causal factors can be modeled using geo-statistical methods and techniques. Ecological niche models are defined as methods that use occurrence data in conjunction with environmental data in order to create a model of the environmental conditions that meet a species' ecological requirements and predict the relative suitability of habitat (Warren and Seifert, 2011). Joseph Grinnell (1917) originated the concept of ecologic niches as the set of conditions under which the species can maintain populations without immigration of individuals from other areas.

The presence of a species indicates that environmental features have allowed that population to be maintained and the interactions with other species have allowed for the species to survive (Hirzel et al., 2008, Soberon 2007). The distribution of a species is the result of interactions among abiotic conditions, biotic conditions, and current dispersal ability and

opportunity for dispersal and colonization (Hutchinson, 1978; Waltari et al., 2007). Ecological niche models can be used to identify potential distribution areas on any landscape and represent a powerful tool for characterizing ecologic and geographic distributions of species across real-world landscapes. Several approaches have been used to model species' fundamental ecological niches.

ENMs have been utilized by researchers to characterize the ecological distribution of species, to identify the habitats of undocumented diseases, and thus to anticipate the areas at high risk for disease occurrence. In some cases, details about ecological features associated with occurrence of disease or species involved in disease transmission may be unclear. ENMs make it possible to relate known occurrences of diseases to GIS layers to generate a quantitative view of what is known about the relationship of disease/species and environmental variation (Peterson, 2006). ENM models have resulted in the ability to predict not only the distribution of vector populations that directly relate to transmission of pathogens, but also disease events and their impact on animal and human health. A constraint in determining the geographic distribution of a species is the availability of data. Field sampling can often be difficult due to cost and terrain limitations. Absence data is even more difficult to obtain due to the hindrance of determining when a species does not exist in an area. But when data are available, ENMs can provide accurate information of a species ecological niche based on environmental characteristics of known species locations (Guisan and Thuiller, 2005; Mak et al., 2010).

When using ENMs approaches in epidemiology, it is necessary to keep in mind that disease processes are dynamic and take place on diverse scales of space and time. They are the products of interactions among pathogens, reservoirs, vectors, etc., and their ecological and distribution dynamics may differ from one scenario to another. Such interactions may vary especially or even vary as a function of interactions with other species (Peterson, 2008). Using such models, decision makers can prioritize not only the determinant factors of the disease, but

also the actions and regulations required for fighting the disease. ENM's have been used in modeling distribution of diseases, such as West Nile virus, plague, and vectors, such as *Anopheles gambiae*, *Phlebotomus papatasi*, *Triatoma infestans* and to examine the distribution and potential distribution of *Lutzomyia* spp. vectors of leishmaniasis in South America (Colacicco-Mayhugh et al., 2010; Gurgel-Gonçalves et al., 2012; Holte et al., 2009; Larson et al., 2008; Levine et al., 2004; Peterson et al., 2005; Sanchez et al., 2015).

Many methods are now available to model a given species/disease niche and, by extrapolation, their geographic ranges. One such method is the Maximum Entropy method or MaxEnt, which is a general-purpose approach for making predictions or inferences involving presence-only datasets (Phillips et al., 2006).

2.3.1. Maximum Entropy Method (MaxEnt)

Maxent is a presence-only niche modeling technique, used to identify the potential distribution of species. This technique represents a great potential tool for identifying distributions and habitat selection of species given its reliance on only presence locations. Usually, analyses are conducted with presence or absence data, but absence data, as mentioned previously, is often unavailable. Maxent is based on a machine learning response that is designed to make predictions from incomplete data (Baldwin, 2009; Phillips et al. 2006).

This method estimates distributions of species by finding the most uniform distribution of sampling points compared to background locations given the constraints derived from the data (Phillips et al. 2006). MaxEnt has emerged as just as reliable a technique for predicting species' distributions as genetic algorithms (GARP) and regression trees (Graham et al., 2008; Phillips, 2009; Wisz et al., 2008). However, the advantage of maximum entropy methods over both genetic algorithms and regression methods is that MaxEnt predicts relative probabilities of occurrence rather than the simple presence or absence of a species. This permits a finer

assessment than what can be achieved from presence-absence predictions alone (Elith et al., 2006).

A major advantage of Maxent is the production of a spatially continuous result, allowing finer detail and visually intuitive outputs. It is not necessary to create a surface map by interpolation of results as using other methods would require (by interpolating results spatially) (Zeimes et al., 2012). The occurrence model building in Maxent starts from a uniform distribution of probability for each cell of the study area. Then, it improves the model iteratively until the gain becomes saturated. The gain is a likelihood statistic that maximizes the probability of presence according to the background data. All dependent variables are used simultaneously (Zaimes et al., 2012).

Maxent is a method that has been frequently used and well documented for developing models for species (biological diversity) as well as for endemic diseases (Du et al., 2014; Holte et al., 2009; Mischler et al., 2011; Murray et al., 2011; Slater and Michael, 2012). A percentage of randomly selected occurrence points can be set aside to be used for testing the accuracy of the model (testing points) and the remaining to be used for building the model (training points) (Masuoka et al., 2009).

Models generated in Maxent can be evaluated by the area under the Receiver Operating Characteristic curve (AUC) and Jackknife analysis. The AUC has been used extensively in the species' distribution modelling literature, and measures the ability of a model to discriminate between sites where a species is present, versus those where it is absent (Hanley and McNeil 1982). The AUC ranges from 0 to 1, where a score of 1 indicates perfect discrimination, a score of 0.5 implies predictive discrimination that is no better than a random guess, and values lower than 0.5 indicate performance worse than random (Engler et al., 2004).

Jackknife analysis evaluates the contribution of each environmental variable to the model providing response curves showing the influence of a variable on the probability of presence. The evaluation is performed in three ways; exclusion of each variable in the run, with

each variable in isolation, and by using all variables. The result is a series of plots of the prediction changes as each environmental variable changes (Murray et al., 2011; Phillips 2004; Zeimes et al., 2012). MaxEnt also facilitates the identification and interpretation of non-linear responses and appears to be better able to fit occurrences of species without over predicting the area in which they are able to live (Phillips et al. 2004; Elith et al. 2006; Sánchez-Flores et al. 2007.

Maxent has been used previously for characterizing the distribution and ecology of vectors of leishmaniasis. Maxent has been used in studies reported in this dissertation as a tool providing valuable understanding of sand fly ecology and the epidemiology of leishmaniasis (Abdel-Dayem et al., 2012; Colacicco-Mayhugh et al., 2010; Salomón et al., 2012).

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Chapter 3: An Ecological Niche Model for Leishmaniasis in Brazil Based on Environmental and Socioeconomic Risk Factors

3.1. Introduction

Leishmaniasis is an infectious parasitic disease, caused by intracellular protozoans of the genus *Leishmania*. In its different presentations, it is estimated that 12 million people are infected and that 2 million new infections occur each year. Up to 350 million people are at risk in 88 countries around the world (WHO, 2010). The global burden is increasing and the prospects for control are still highly dependent on research progress to obtain better tools and a more cost-effective strategy for case management and vector control (Desjeux, 2004).

In Brazil, this endemic disease takes two forms that are reflected in the clinical symptoms: visceral and cutaneous leishmaniasis. The two forms of disease represent a serious public health concern due to their complexity and resistance to control efforts. They belong to the group of neglected tropical diseases (NTD), intrinsically associated with poverty, health inequities and dynamic environmental features (Boelaert et al., 2000; Camargo and Lagoni, 2006; Sherlock, 1996). Visceral leishmaniasis (VL) in Brazil is caused by *Leishmania infantum* and transmitted by the bite of the sand fly *Lutzomyia longipalpis* (Brasil, 2006; Umakant, 2008). Cutaneous leishmaniasis (CL) has a variety of causal agents, reservoirs and vectors with different transmission patterns. Out of the 12 new world species of *Leishmania* known to cause CL in humans, seven can be found in Brazil, resulting in an endemic CL problem greater than in any other Western country (Ashford, 1996). The three main species that cause disease in humans in Brazil are *Leishmania Viannia braziliensis*, *Leishmania Viannia guyanensis* and *Leishmania Leishmania amazonensis*. The principal vector species involved in transmission of CL are *Lutzomya flaviscutellata*, *L. whitmani, L. umbratilis, L. intermedia, L. wellcome and L. migonei* (Brasil, 2007).

Even though these diseases are not ranked among the leading infectious diseases in terms of global burden, they assume great medical, social, and economic importance in endemic areas. These diseases have an ample distribution and cases of both forms of

leishmaniasis have been reported in all Brazilian regions with varying transmission patterns depending on the region of occurrence. The sand fly vectors are present in a variety of habitats and both vectors and reservoir hosts are affected by variation in rainfall, vegetation, temperature, and relative humidity (Elnaiem et al., 2003).

In Latin America, VL has been reported in at least 12 countries, but Brazil has the majority of cases (90%), concentrated especially in the Northeast region (Albuquerque et al., 2012). Visceral leishmaniasis is reported in 19 out of 27 states of Brazil in which approximately 1,600 municipalities reported autochthonous transmission (Brasil, 2006; Harhay et al., 2011). Cutaneous leishmaniasis is distributed worldwide and in the Americas there are records of cases from the extreme south of the United States to the north of Argentina, with the exception of Chile and Uruguay. In Brazil CL has been reported in all states with an incidence of about 20,000 new cases per year in 2008 (Brasil, 2007; Brasil, 2009).

Control programs currently in place have not yet shown the desired effect of reducing the incidence of VL or CL in Brazil. Thus, considering the difficulties in controlling these diseases, new methodologies to better define transmission dynamics and risk areas has become a necessity.

Factors affecting the spatial distribution of pathogens, hosts and vectors, and the probability of coincidence in an area are fundamentally important to understand disease dynamics. Environmental changes and ecological disturbances exert an influence on the emergence and proliferation of zoonotic parasitic diseases by altering the ecological balance and context within which vectors and their parasites breed, develop, and transmit disease, thus resulting in strong spatial patterns of risk and incidence (Patz et al., 2000). Both environmental-climatic factors and the socioeconomic complex associated with these diseases are of great importance. The transition from rural to urban settings is a phenomenon believed to be the result of infected individuals and canines moving to larger cities and this has been well characterized for VL although the eco-epidemiological complex which characterizes VL in

endemic areas is not yet well understood (Costa et al., 1990; Gontijo and Melo, 2004; Werneck et al., 2007). For CL, the transmission pattern is centered on people who work or live within tropical forests. However, when comparing current patterns of occurrence, it has been suggested that the behavior of vectors may be changing, perhaps in response to environmental shifts (Alessi et al., 2009; Brasil, 2006; Brasil, 2009; Donalisio et al., 2012). The association of leishmaniasis with poverty can be related to inadequate housing conditions that provide breeding sites for the vector and access to canine and other reservoir hosts, especially in transition areas of agriculture/forest/urban-environments.

A growing number of reports have shown that remote sensing (RS) and geographic information systems (GIS) are important tools for generating predictive maps which contribute to a better understanding of the ecological factors affecting parasitic diseases (Bavia et al., 2005; Colacicco-Mayhugh et al., 2010; Eisen and Eisen, 2011; Foley et al., 2008). Although RS products do not identify the parasites and vectors themselves, they can be used to characterize the environment in which they thrive. The advantages of RS over ground measurements are that they can be collected repeatedly, automatically, and they are considerably faster to obtain. Studies have shown that it is possible to use climate data, environmental data and socioeconomic data, to better understand the dynamics of endemic leishmaniasis and that such information represents a valuable tool in predicting future outbreaks (Carneiro et al., 2004; Carneiro et al., 2007; Cerbino-Neto et al., 2009; Gonzáles et al., 2010), although ecological/socioeconomic determinants have not been fully elucidated. The combination of RS, GIS technologies and ecological niche models (ENM) presents an alternative approach (Foley et al., 2008; Gonzáles et al., 2010; Abdel-Dayem et al., 2012). ENM incorporate both the ecological requirements and spatial locations of species and predict potential species occurrences in a defined area based on known distributions, thus providing useful information for control programs. Maxent ENM software utilizes presence or absence data to predict probability distribution based on the maximum entropy method (Phillips et al., 2006) and has

been used to define suitable areas for a number of species and diseases (Elith et al., 2006; Holt et al., 2009; Quintana et al., 2013).

The goal of this study was to identify environmental and socioeconomic factors associated with the occurrence of CL and VL in Brazil in the years 2005 to 2009, using RS, GIS and ENM techniques to predict the risk of disease at the municipality level.

3.2. Material and Methods

The territorial extent of Brazil is 8,502,728.269 Km^2 with 27 states and a total of 5,565 municipalities. The latest National Census counted approximately 190,755,799 inhabitants with an estimated population density of 22.43 inhabitants/Km^2 (IBGE, 2010).

A GIS database was constructed using records of VL cases by municipality in Brazil during 2005-2009 available online in the Brazilian national notifiable diseases information system (SINAN) database. The socioeconomic database included data from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (PAHO), also at the municipality level. The variables obtained for this study from IBGE were population, poverty incidence, gross domestic product (GDP), gross domestic product per capita, percentage of households with piped water, percentage of households with regular garbage collection, percentage of households with sewage system, number of health facilities, literacy rate, human development index (HDI), infant mortality rate and unemployment. Variables obtained from PAHO data included unsatisfied basic needs for people (UBNp), education, overcrowding, subsistence, sanitation, and unsatisfied basic needs for housing (UBNh), plumbing, sanitation, drinking water and electricity availability.

The environmental database was constructed included long-term-normal monthly climate data at 1km spatial resolution from WorldClim (http://www.worldclim.org, last accessed October 19, 2010) on mean annual temperature, minimal annual temperature, maximal annual temperature, precipitation, derived bioclimatic data (Bioclim 1-19,

http://www.worldclim.org/bioclim last accessed October 19, 2010), altitude, and MODIS remote sensing annual composite image data for 2005-2009 on land surface temperature (day and night) enhanced vegetation index (EVI) and normalized difference vegetation index (NDVI).

Statistical analyses performed included correlation between incidence of the disease (per 10,000 inhabitants) against each variable in the database. Variance inflation factor coefficients (VIF) were used to remove some variables, based on co-variance of individual variables that may influence results. After variables with VIFs greater than 10 were removed, a stepwise linear regression with the variables that were correlated with the disease and logistic regression was performed. All statistical analyses were performed using SAS/STAT® software, version 9.1.3 of the SAS system for Windows (SAS, 2004).

WorldClim, Bioclim and MODIS data were used to generate a probability surface for CL and VL based on environmental features using Maxent. A similar approach was used to generate a model from the socioeconomic database. Separate models were executed for environmental and socioeconomic data and prediction models for occurrence of VL and CL in Brazil were created. For each model generated, 25% of the VL and CL point case records were randomly selected to evaluate the models. Only presence of disease was analyzed and accuracy of the models was assessed using the area under the receiver operating characteristic (ROC) curve (AUC). AUC values up to 0.5 indicate random occurrence of leishmaniasis in the study area, whereas values close to 1.0 indicate a statistically perfect discrimination of occurrence (Engler et al., 2004). Prediction power of variables was evaluated by Jacknife analysis. Maps of the spatial distribution of VL and CL and prediction model surfaces were created using ArcMap version 10.1 (ESRI, 2011). A general schematization of model development is depicted in Figure 3.1.

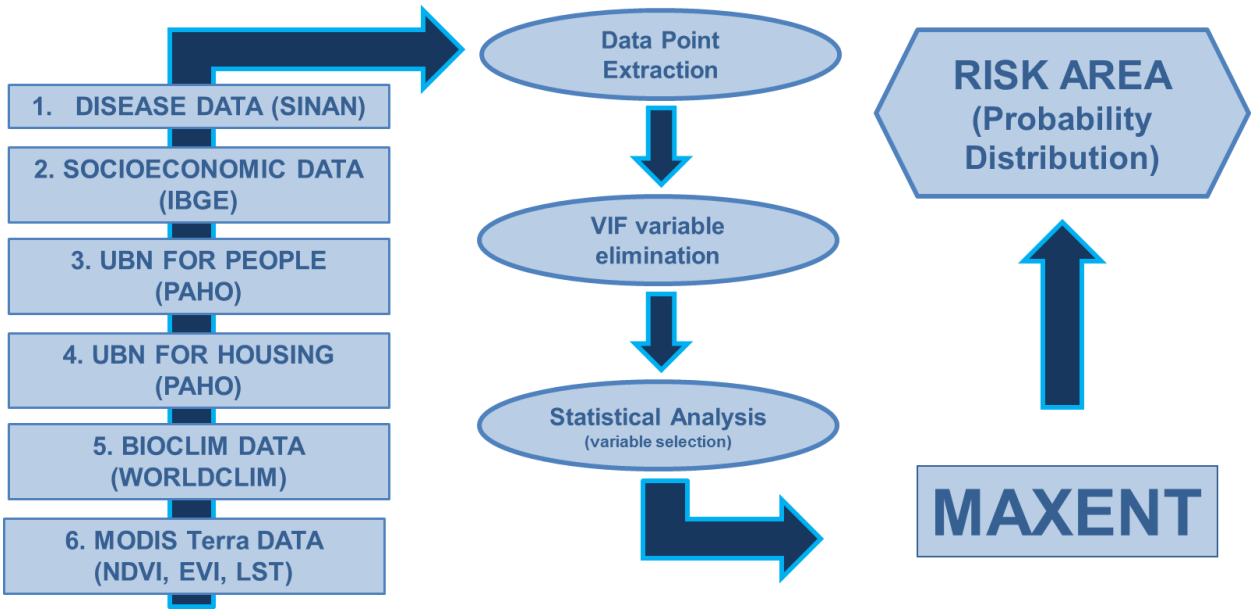

Figure 3.1.Schematization of Ecological Niche Model for Brazil - Development of models for CL and VL in Brazil using Maxent and socioeconomic and environmental variables.

3.3. Results

The socioeconomic indicators evaluated in this study revealed that approximately 0.11% of the municipalities in Brazil did not have health units and those areas did not report any leishmaniasis cases during the period of study. Regarding urban-infrastructure, it was observed that all the households in the municipalities studied have some form of sanitation system in place. Approximately 0.43% of the households did not have piped water available and 0.61% of the households did not have garbage collection. Four percent of the municipalities had HDI equal to zero and 0.25% of the municipalities reported households with no income. Water source seemed to be a convergence factor present in all the analysis performed. The odds for the occurrence of either CL or VL did not increase regarding the water source but water seemed to behave as a protecting factor.

3.3.1. Cutaneous Leishmaniasis

During 2005 to 2009, a total of 96,351 cases of CL were registered at SINAN, affecting 13% of the municipalities of Brazil (Figure 3.2). The state with the highest number of cases reported was Pará (18.54%) and the municipality with the highest number of records was Manaus (4.25%), which is located in the state of Amazonas. CL was reported in 846 municipalities, with the majority of cases in the North and Northeast regions (44.7 and 27.46% respectively) (Table 3.1).

Pearson's correlation showed that number of health units per municipality ($p<0.0001$), literacy rate ($p=0.021$), water source ($p=0.038$) and UBNh sanitation ($p=0.02$) were the variables positively correlated with CL incidence. For the regression model, the variables that best explained CL incidence were number of health units, water source, garbage collection, literacy rate and UBN education ($p<0.0001$; $r^2=0.4985$).

The Maxent model for socioeconomic factors showed that the most influencing variables in the model were UBNh sanitation, GDP per capita and literacy rate with 55%, 21% and 19% of influence respectively (AUC=0.795; $p=0.0194$). UNBh sanitation was the variable that, when added and omitted in the model, provides the most information in the model for CL (Figure 3.3).

For the environmental analysis, Pearson's correlation showed that CL incidence was inversely correlated with temperature seasonality ($p<0.0001$), and directly correlated with mean temperature of coldest quarter, precipitation in wettest month, precipitation in wettest quarter ($p<0.0001$) and annual precipitation ($p=0.03$). The regression model showed isothermality, temperature annual range, annual precipitation and NDVI as the best explanatory variables ($p=0.03$; $r^2=0.04$). Annual precipitation seemed to be a convergence factor present in all the analysis performed; however, the odds for the occurrence of CL remained the same regarding this variable.

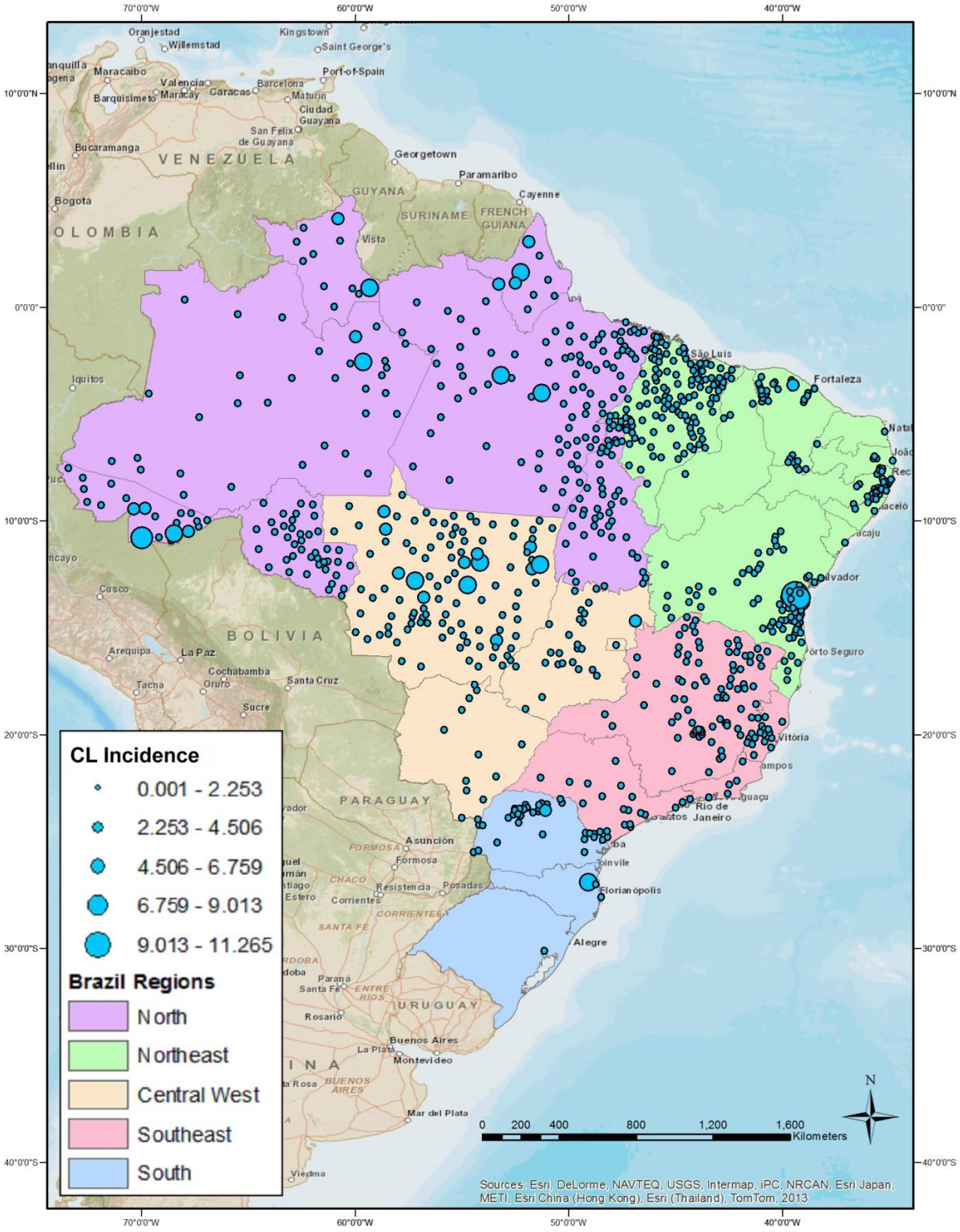

Figure 3.2. Spatial Distribution of CL Cases Reported in Brazil from 2005 to 2009 by Geographic Region - Incidence was calculated per 100.000 inhabitants.

Table 3.1. Cases of Cutaneous Leishmaniasis Reported by State.

Region	State		CL cases
North	Acre	AC	5,102
	Amazonas	AM	8,492
	Amapá	AP	2,878
	Pará	PA	17,863
	Rondônia	RO	5,415
	Roraima	RR	1,554
	Tocantins	TO	1,761
Northeast	Alagoas	AL	289
	Bahia	BA	11,181
	Ceará	CE	4,545
	Maranhão	MA	9,599
	Paraíba	PB	195
	Pernambuco	PE	1,695
	Piauí	PI	604
	Rio Grande do Norte	RN	31
	Sergipe	SE	16
Central West	Distrito Federal	DF	244
	Goiás	GO	1,035
	Mato Grosso	MT	14,746
	Mato Grosso do Sul	MS	323
Southeast	Espírito Santo	ES	358
	Minas Gerais	MG	5,112
	Rio de Janeiro	RJ	588
	São Paulo	SP	1,018
South	Paraná	PN	1,481
	Rio Grande do Sul	RS	13
	Santa Catarina	SC	213
Total			**96,351**

The Maxent environmental model using WorldClim and MODIS data showed that variables that most contributed to the prediction model were precipitation in September (26.2%) and annual precipitation (17.3%) (AUC=0.80; $p<0.0001$).

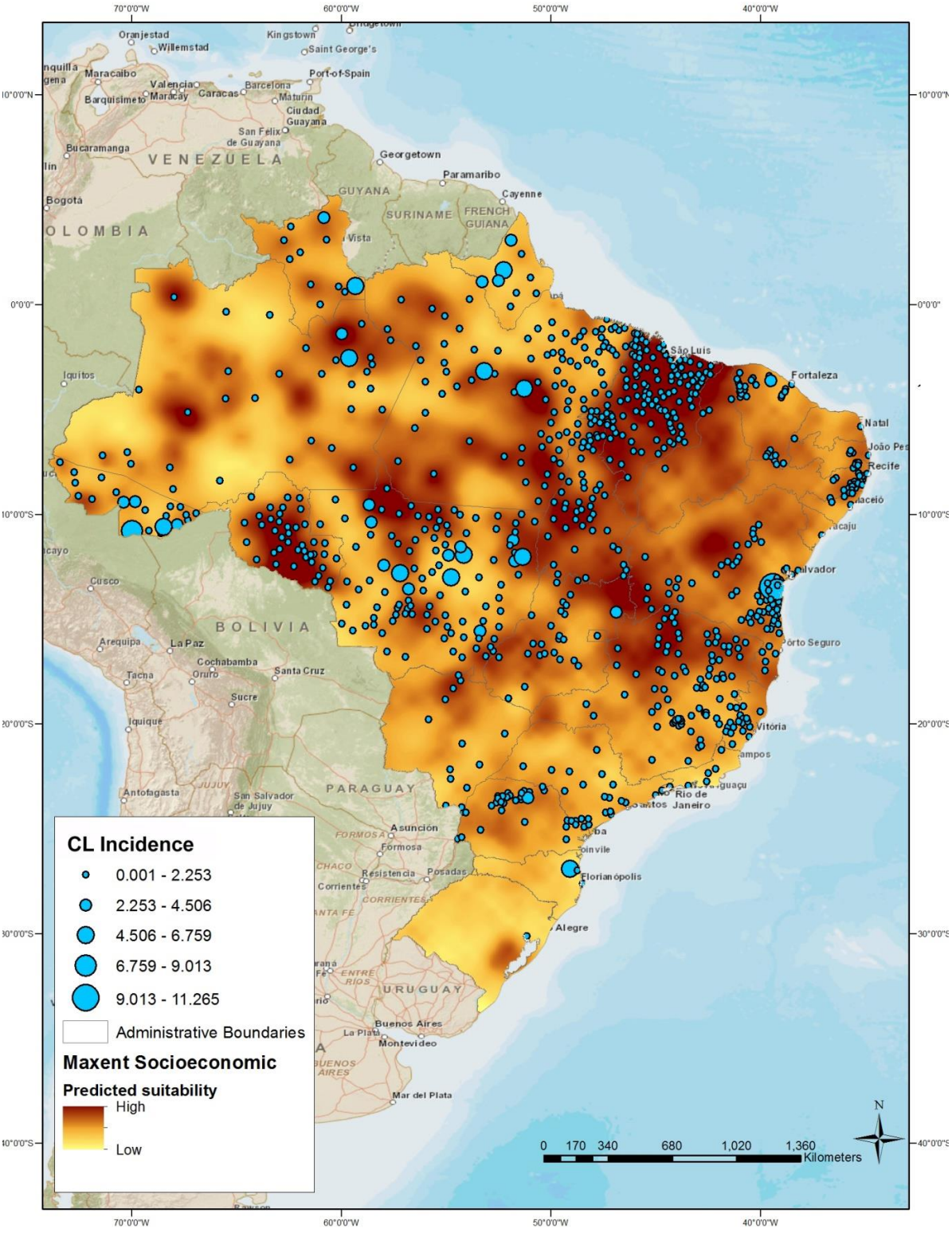

Figure 3.3. Predicted Suitability Map for the Incidence of CL Considering Socioeconomic Factors - Darker areas represent higher probability of cutaneous leishmaniasis in relation to poor sanitation, lower income and illiteracy.

The environmental variable with highest gain, when used in isolation, was precipitation in September, which appeared to have the most useful information by itself. The environmental variable that appears to have the most information that isn't present in the other variables was annual precipitation. A summary of all the important variables and their significance in both, socioeconomic and environmental models for CL is shown on Table 3.2. A probability distribution map for CL in Brazil according to the environmental variables is shown in Figure 3.4.

Table 3.2. Summary of Socioeconomic and Environmental Analysis for CL - Importance of variables and their significance are shown.

Model	Analysis	Variable	P value
Socieconomic	Pearson's correlation	Number of health units	p<0.0001
		Literacy rate	p=0.021
		Water source	p=0.038
		UBNh sanitation	p=0.02
	Regression	Number of health units	p<0.0001; r^2=0.4985
		Water source	
		Garbage collection	
		Literacy rate	
		UBN education	
			AUC=0.795; p=0.0194
	Maxent	UBNh sanitation	55% contribution
		GDP per capita	21% contribution
		Literacy rate	19% contribution
Environmental	Pearson's correlation	Temperature seasonality	p<0.0001
		Mean temp of coldest qt	p<0.0001
		Precip of wettest month	p<0.0001
		Precip of wettest qt	p<0.0001
		Annual precipitation	p=0.03
	Regression	Isothermality	p=0.03; r^2=0.04
		Temperature annual range	
		Annual precipitation	
		NDVI	
			AUC=0.80; p<0.0001
	Maxent	Precipitation in September	26.2% contribution
		Annual precipitation	17.3% contribution

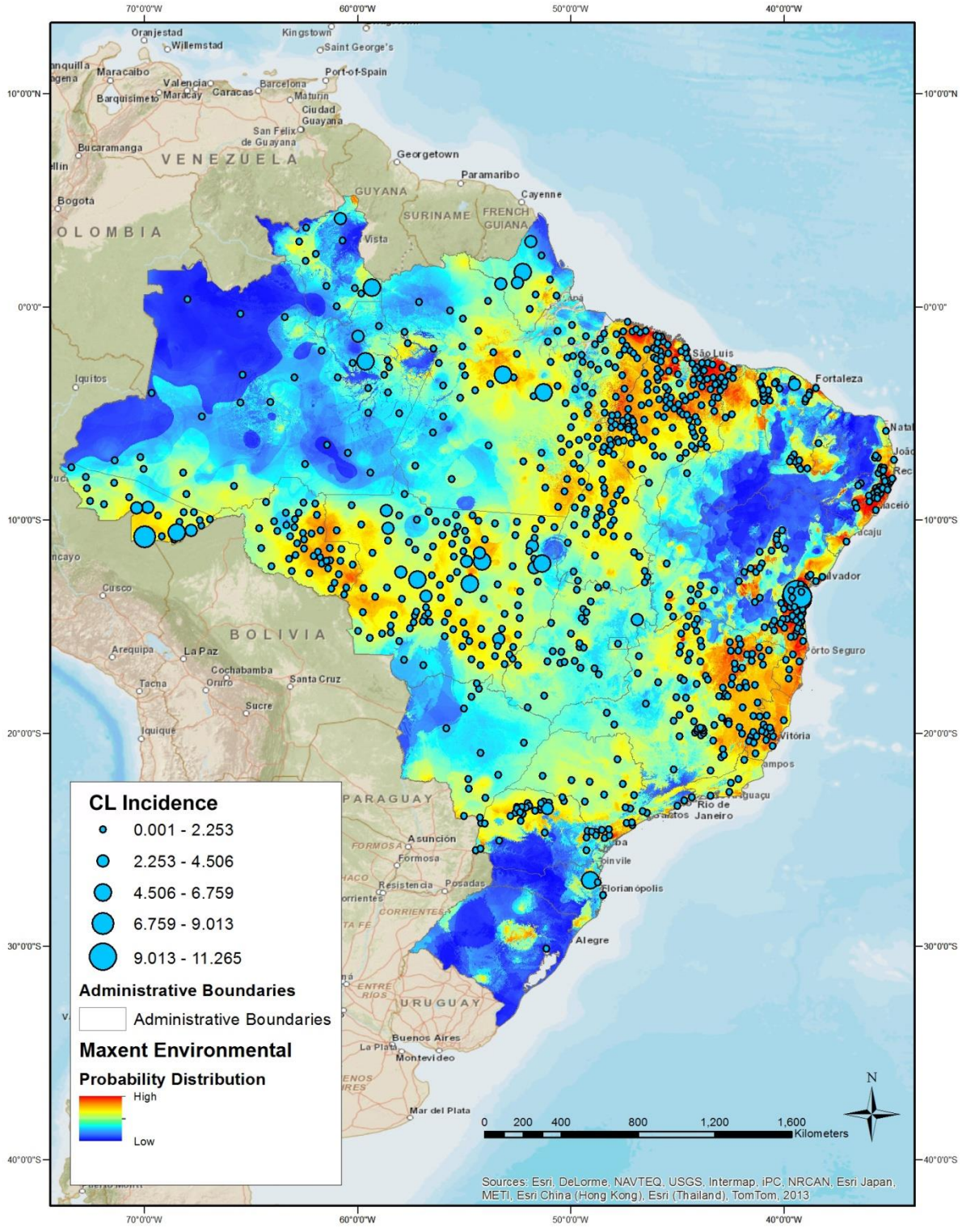

Figure 3.4. Predicted Suitability Map for the Incidence of CL Considering Environmental Factors - Red areas represent higher probability of cutaneous leishmaniasis in relation to precipitation in September and annual precipitation.

3.3.2. Visceral Leishmaniasis

During 2005 to 2009, a total of 13,563 cases of VL were registered at SINAN, which corresponds to 2.2 % of the municipalities of Brazil (Figure 3.5).

The state with the highest number of VL cases observed was Ceará (15.55%) and the municipality with the highest number of cases observed was Teresina (10.43%), which is located in the state of Piauí. VL was reported in 122 municipalities, with the majority of the cases concentrated in the North and Northeast regions (21.31 and 47.42 % of the cases respectively) (Table 3.3).

Pearson's correlation analysis showed that VL incidence was positively correlated with the number of health facilities, water source and garbage collection ($p<0.0001$). The linear regression analysis showed garbage collection, literacy rate and water source as the socioeconomic indicators that best explain the incidence of VL ($p<0.0001$; $r^2=0.414$).

The Maxent socioeconomic model showed that UBNh drinking water availability (49%), poverty and literacy (19.7%) were the most important variables for VL (AUC=0.889; $p=0.015$). Drinking water availability is the variable that both added and omitted the most information from the VL model. A probability distribution map for VL in Brazil based on the Maxent environmental variables included in the analysis is shown in Figure 3.6.

For environmental data, the only correlation observed between VL incidence and environmental features was an inverse correlation with temperature seasonality ($p<0.0001$). That was also the only predictor variable in the regression models ($p=0.043$; $r^2=0.42$).

The Maxent environmental model using WorldClim and MODIS data showed that variables that most contributed to the VL prediction model were precipitation seasonality (14.5%) and precipitation in October (11.6%) (AUC=0.948; $p<0.0001$). The environmental variable with highest gain when used in isolation was precipitation for October, which appears to have the most useful information by itself.

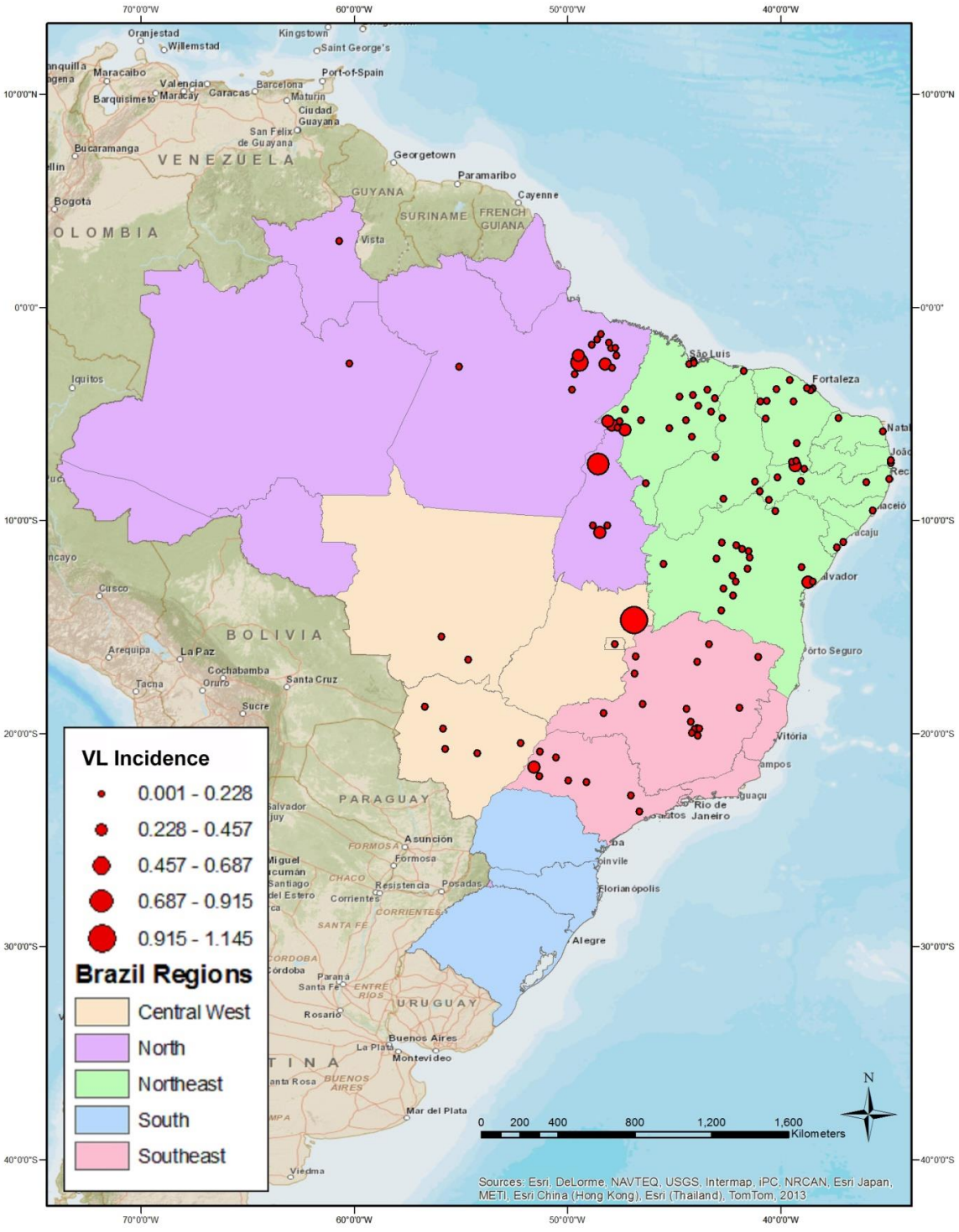

Figure 3.5. Spatial Distribution of VL Cases Reported in Brazil from 2005 to 2009 by Geographic Region - Incidence was calculated according to number of cases per 100.000 inhabitants.

Table 3.3. Cases of Visceral Leishmaniasis Reported by State.

Region	State		VL cases
North	Acre	AC	0
	Amazonas	AM	13
	Amapá	AP	0
	Pará	PA	1,414
	Rondônia	RO	0
	Roraima	RR	22
	Tocantins	TO	1,441
Northeast	Alagoas	AL	153
	Bahia	BA	559
	Ceará	CE	2,109
	Maranhão	MA	1,242
	Paraíba	PB	72
	Pernambuco	PE	311
	Piauí	PI	1,520
	Rio Grande do Norte	RN	296
	Sergipe	SE	171
Central West	Distrito Federal	DF	325
	Goiás	GO	86
	Mato Grosso	MT	115
	Mato Grosso do Sul	MS	1,026
Southeast	Espírito Santo	ES	0
	Minas Gerais	MG	1,876
	Rio de Janeiro	RJ	0
	São Paulo	SP	812
South	Paraná	PN	0
	Rio Grande do Sul	RS	0
	Santa Catarina	SC	0
Total			**13,563**

The environmental variable that decreases the gain the most when it is omitted is precipitation seasonality, which appears to have the most information that isn't present with the other variables. A summary of all the important variables and their significance in both, socioeconomic and environmental models for VL is shown on Table 3.4. A probability distribution map for VL in Brazil according to the environmental variables is shown in Figure 3.6.

Table 3.4. Summary of Socioeconomic and Environmental Analysis for VL - Importance of variables and their significance are shown.

Model	Analysis	Variable	P value
Socieconomic	Pearson's correlation	Number of health units	$p<0.0001$
		Water source	$p<0.0001$
		Garbage collection	$p<0.0001$
	Regression	Garbage collection	$p<0.0001$; $r^2=0.414$
		Literacy rate	
		Water source	
			AUC=0.795; p=0.0194
	Maxent	UBNh drinking water	49% contribution
		Poverty rate	19.7% contribution
		Literacy rate	19.7% contribution
Environmental	Pearson's correlation	Temperature seasonality	$p<0.0001$ (- corr.)
	Regression	Temperature seasonality	$p=0.043$; $r^2=0.42$
			AUC=0.80; p<0.0001
	Maxent	Precipitation seasonality	14.5% contribution
		Precipitation in October	11.6% contribution

3.4. Discussion

The Ministry of Health records VL and CL cases in Brazil at the municipality level and uses the mean number of human cases over a period of five years to identify areas of intense and moderate transmission and to indicate where currently recommended control measures should be prioritized.

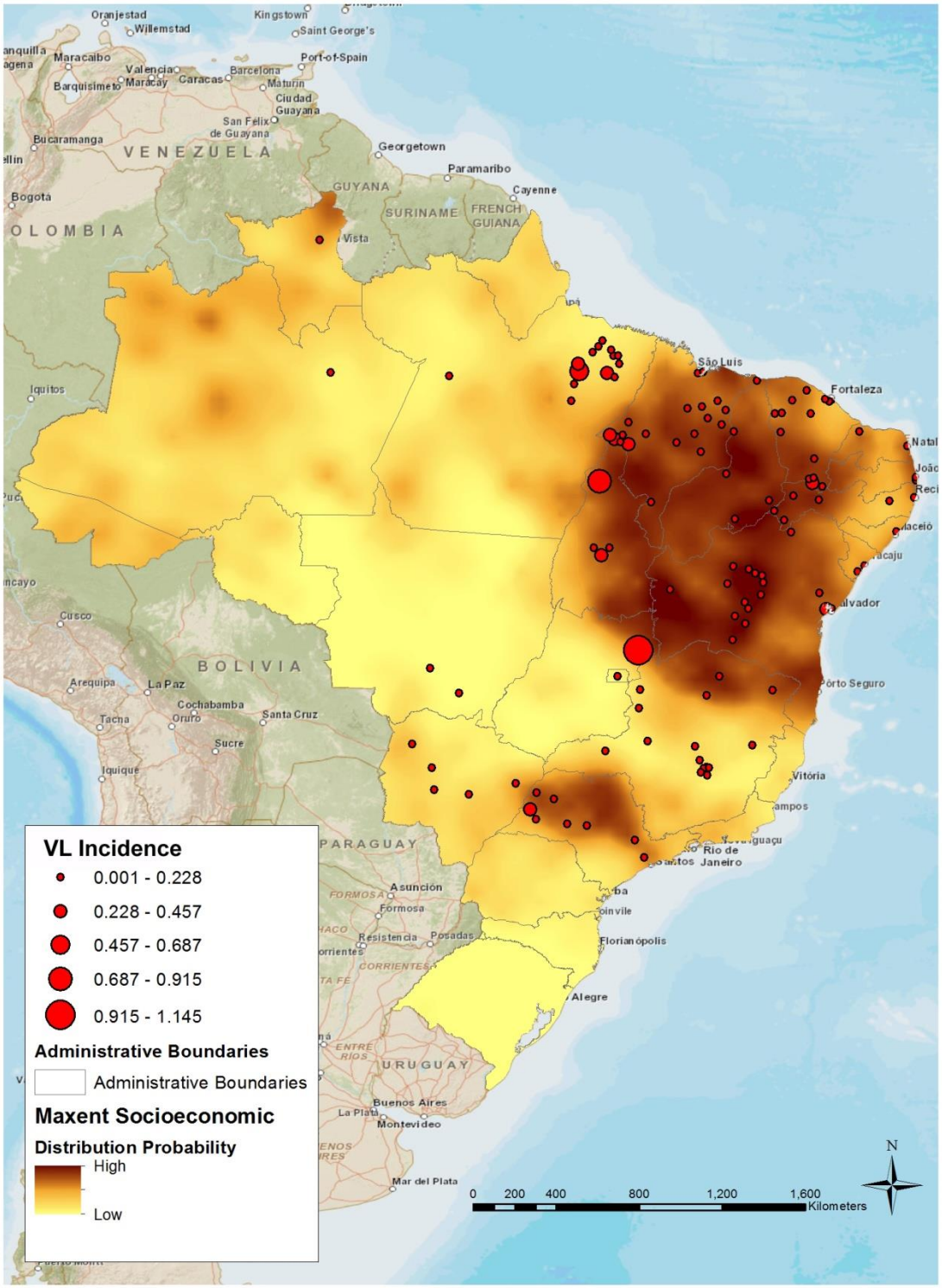

Figure 3.6. Predicted Suitability Map for the Incidence of VL Considering Socioeconomic Factors - Darker areas represent higher probability of visceral leishmaniasis occurrence in relation to water availability, poverty and literacy rates.

Although only 15.94% of the municipalities reported some form of leishmaniasis, the absence of this endemic disease in some municipalities could be due to the lack of health services, leading to non-reporting of the disease in some cases or notification reported in a different municipality where health care was more accessible. Moreover, municipalities with better access to health services may be intensifying their control policies; therefore, reports of diseases may be higher in such locations due to more infected people being diagnosed (Bavia et al., 2011; Brasil, 2006).

3.4.1. Socioeconomic Model

Infrastructure services can be an indicator of the urbanization and quality of sanitation of an area, and indirectly provide information about proliferation of vectors and presence of animals near residences. Cerbino-Neto et al. (2009) observed that high incidence rates of VL were associated with the percentage of households with piped water and population growth. Unsatisfactory housing conditions were associated with higher rates of VL in studies by Santana et al. (2009); in that study the majority of the households had piped water available (71%) but lacked garbage collection (71%). Garbage accumulation provides food and shelter conditions for wild and stray animals that begin to reside in proximity to humans. This behavior elucidates how VL is a disease of intimate relationship with the social conditions to which individuals are subjected (Ferro et al., 1995; Nascimento et al., 1996). Correia et al. (2005), by contrast, found that neither water nor garbage collection services had a significant relationship with VL in Teresina, Piaui state, Brazil.

The socioeconomic model generated in Maxent, showed that human development index (HDI), poverty, and literacy rate were the most important variables in the prediction model for VL. Borges et al. (2008) observed that an illiterate person is eight times more likely to acquire VL than a literate person. Low literacy rates are intrinsically associated with poverty and low HDI scores and indirectly lead to low sanitation conditions.

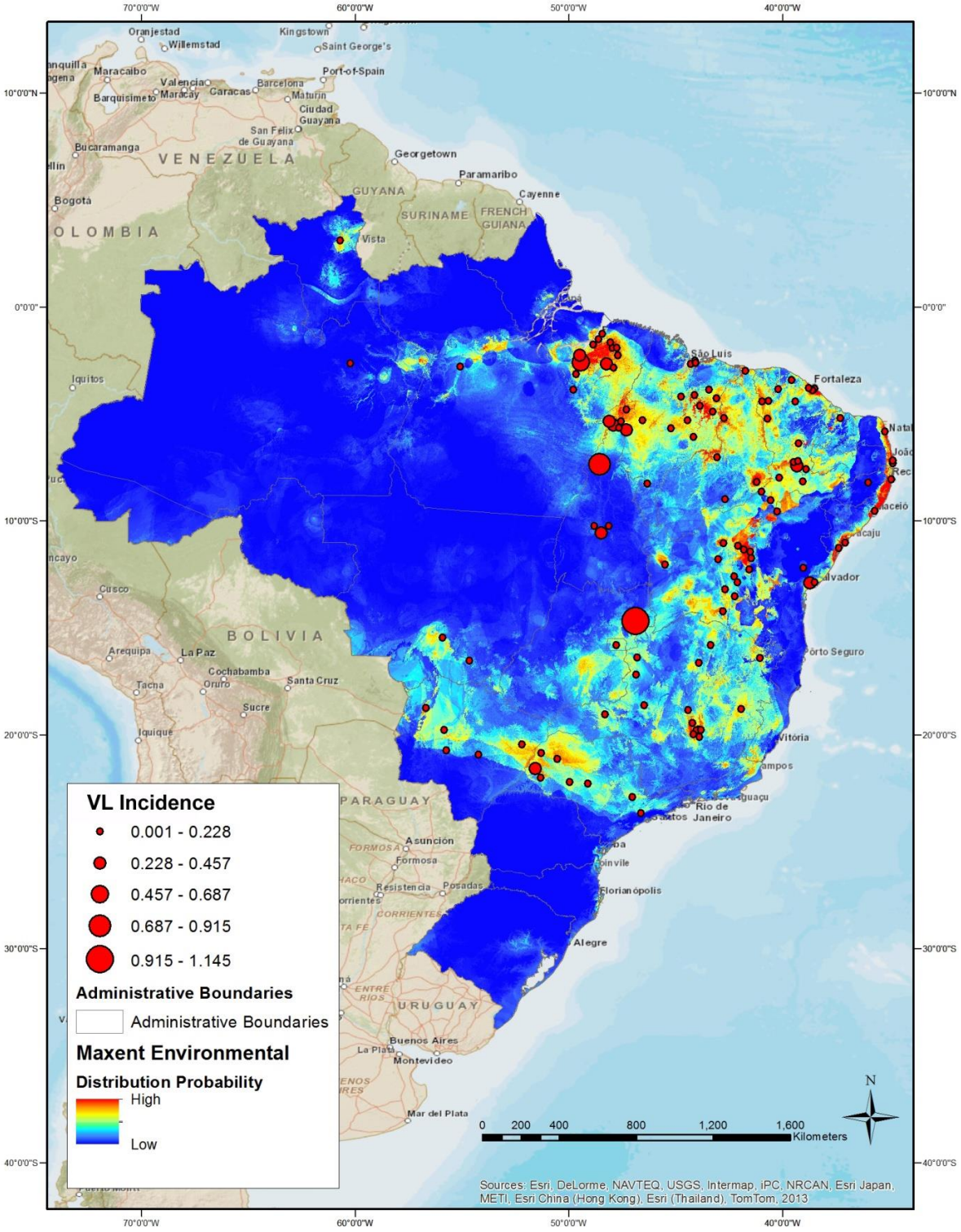

Figure 3.7. Predicted Suitability Map for the Incidence of VL Considering Environmental Factors - Red areas represent higher probability of visceral leishmaniasis in relation to precipitation seasonality and precipitation pf October.

The results in this study agree with the literature consensus that literacy/education plays an important role in the occurrence of leishmaniasis. Poor socio-economic conditions have an amplifying effect on the association between canine infection and the incidence of human VL. In areas of low socio-economic standing, high or rising rates of infection in dogs were predictive of high rates of human disease, due to the degree of contact among susceptible people, infectious dogs, and infected vectors being greater in impoverished areas than in wealthy areas, even if the incidence of infection in dogs is similar in both areas (Werneck et al., 2007).

Several studies have shown that both CL and VL tend to be more predominant in populations with low literacy rate, low income, precarious sanitation and habitation (Almeida and Werneck, 2014; Borges, 2006; Cerbino-Neto, 2009; Costa et al., 2005; Oliveira and Maciel, 2003). However, similar studies have also suggested that leishmaniasis, in particular VL, is not confined to pockets of poverty, or low socioeconomic status. This could be the result of having the domestic dog as the primary companion animal in the household, a behavior observed amongst Brazilians of all social levels (Carmo, 2002; Marcelino, 2007).

Sampaio and Paula (1999) observed that in the Federal District of Brazil, the majority of patients with CL migrated from another state and were living in inadequate sanitation conditions. However, other authors found that the distribution of human and canine VL did not have a direct relationship with the socioeconomic level of the population making the role of socioeconomic factors in the occurrence of VL unclear (Correia et al., 2005; Marcelino, 2007).

Although the percentage of municipalities lacking basic infrastructure was less than 1%, the poverty situation in which these people might be living suggest a lack of acquiring and applying protective measure such as bed nets, thus increasing exposure to the vectors. The success of control strategies is highly dependent on resource availability, knowledge and attitude of inhabitants at risk of facing endemics (Santos et al., 2000). Pardo et al. (2006), while applying a questionnaire in Huila, Colombia, observed that 35% of the people interviewed had knowledge about CL and practiced measures directed at its control. The significant association

they found between the knowledge of the sand fly's role in transmission and the practice of CL control is evidence of the impact of population knowledge in health educational campaigns. Such findings suggest that programs focused on population education, besides being potential tools of epidemiology control, can be limited due to the low level of education, which can also interfere with preventive measures (Bevilacqua et al., 2001).

In regard to household infrastructure, Cesse (1999) identified that high incidence of leishmaniasis occurred in neighborhoods deprived of basic infrastructure (sanitation, health units, police, telephone access and schools), however they did have electricity and piped water. Garbage collection was irregular and in most of the cases garbage was just abandoned. The houses were constructed using materials such as cardboard or mud walls. These observations reinforce the theory that a pattern of precarious infrastructure is fundamental to the maintenance of leishmaniasis transmission.

3.4.2. Environmental Model

Climatic factors such as temperature, humidity and precipitation can influence sand fly populations. The introduction of environmental features in epidemiological models is essential too since they may dictate parasite and vectors development (Correia et al., 2005; Dias et al., 2007). Studies have suggested that environmental features could be affected by socio-economic development, considering the levels of neighborhood uniformity and zoning restriction for economic group at the interface with the local environment. The inhabited area is usually characterized by a set of low profile social-economic and sanitary conditions, with many domestic animals in the peridomicile, which favors the occurrence and spread of VL (Cesse, 1999). Recent studies in Europe predicting the effects of temperature increases on VL, were defining risk areas as an effort to prevent the emergence of tropical diseases under global climate and land cover changes (Maroli et al., 2008; Fischer et al., 2010; Molina et al., 2012). Ready (2010) reported the natural spread of *Leishmania infantum* to regions where uninfected

vectors are present as one of the main factors contributing to the risk of reemergence of VL in Europe. Peterson and Shaw (2003) predicted under climate change scenarios, an extension of the geographic range of *Nyssomyia whitmani* in Southeast Brazil, where CL appears to be re-emerging.

3.4.3. MaxEnt Models

Studies on geographic and ecological distribution of vectors and cases of disease offer powerful tools for identifying risk zones and exploring potential interactions between hosts, vectors, and parasites (Peterson and Shaw, 2003). Maxent models have been useful to demonstrate suitable areas for occurrence of insect species (Rangel et al., 2012; Scheingross, 2007) as well as diseases such as blastomycosis (Reed et al., 2008), plague (Holt et al., 2009), chagas disease (Mischler et al., 2012) and leishmaniasis (González et al., 2010). Maxent is a software application that utilizes species presence data to predict its distribution based on the maximum entropy method (Phillips et al., 2006).

Temperature and precipitation were the most influencing variables in the models. CL seemed to be particularly sensitive to oscillations in daily and seasonal temperatures while VL was sensitive to oscillations in rainfall throughout the year. Gonzalez et al. (2010) were able to create future scenarios for leishmaniasis in North America using Maxent and worldclim variables and were able to conclude that changes in climate may exacerbate the ecological risk of human exposure to leishmaniasis in areas north of the present range of the disease in the United States and possibly in parts of south-central Canada. A Maxent study of vectors associated with CL in Argentina, showed precipitation seasonality as one of the variables with most contribution to their ENMs (Quintana et al., 2012). These findings are similar to what it was observed in this present study, however, precipitation seasonality showed the greatest contribution only in the ENM for VL. In contrast, Quintana et al. (2012), observed that annual precipitation did not contribute in their CL models, but it was one of the variables with the most contribution to the

ENMs developed for CL in Brazil, and the variable with the most useful information if omitted from the model. These differences in results observed may be due to climatic differences in these countries as well as the geographic extent of the study.

Ecologic niche modeling and distribution prediction with Maxent as well as other methods (i.e, GARP, regression trees) are being used increasingly to investigate how environmental factors might influence infectious disease transmission. Such models have been used to predict the occurrence areas of sand flies and leishmaniasis in Brazil. Using a Maxent approach, Almeida et al. (2013) identified temperature seasonality and annual mean precipitation as variables that most influenced the distribution of *L. longipalpis* in Mato Grosso State, Brazil. They also identified temperature seasonality and precipitation in the driest month as the variables that most influenced VL cases in the same state. Abdel-Dayem et al. (2012) identified higher suitability for occurrence of *Phlebotomus papatasi*, the vector of CL in Libya, in areas confined to the coast at altitudes lower than 600 m. Precipitation was the most significant predictive power in their models, while temperature and elevation variables were less influential. González et al. (2014), observed *Lutzomyia longipalpis* and *Luzomyia evansi,* vectors of VL in Colombia, to be very sensitive to variations in precipitation. Their climate change projections showed an overall reduction in the spatial distribution of these species, promoting a shift in altitudinal distribution for *L. longipalpis* and confining *L. evansi* to certain regions in the coast. Sanchez et al. (2015), used ENMs and Maxent to predict the distribution of seventeen sand fly species in Venezuela and found that precipitation variations are the variables that most influenced their models, elucidating the importance of climatic factors in vector distribution and consequent disease dispersion. Though vegetation has had an influence on the occurrence of leishmaniasis, climatic factors, specifically precipitation, seem to have a higher influence in the probability distribution of VL and CL in Brazil. Vegetation abundance can be attributed in large part to the influence of climatic factors, therefore its intrinsic association with disease occurrence.

Some authors have used the same Maxent approach, but targeted specific biotic factors such as vegetation biomass and landcover and were able to identify specific areas and seasonal associations with leishmaniasis vectors abundance (Donalisio et al., 2012; Salomón et al., 2012). In the Middle East, ENMs for *Phlebotomus alexandri*, a vector of VL, and *P. papatasi,* a vector of CL, predicted land cover as the most influential environmental layer in model development for both species (Colacicco-Mayhugh et al., 2010). In the state of Bahia, Brazil, Nieto et al. (2006) estimated a higher incidence of VL in hot and dry areas (Caatinga), whereas areas of Cerrado and the Atlantic Forest were considered to be less suitable for disease transmission.

Modelling enables the development of geographic predictions of diseases transmitted by insect vectors that assume important implications for human health (Almeida et al., 2013). Nevertheless, lack of more precise information about transmission patterns and prevention of leishmaniasis, as well as its associated risk factors, still represents an important limiting factor in prevention and control of this endemic disease in Brazil. For a better understanding of the dynamics of this endemic disease, we must consider that the occurrence of leishmaniasis in Brazil may be the result of socioeconomic factors coupled with domestic environmental features.

3.5. Conclusion

Despite the coarse scale of the study at the national level, the socioeconomic variables used in this study showed water source, sanitation and literacy rate as good predictors for disease incidence. Water source is an indicator of unsatisfactory house conditions; sanitation is a indirect measurement of vector and host abundance; literacy rate has been reported in the literature as a factor that can increase the risk of VL by 8 times. Comibined, these factors provide a picture of the household situation where diasese can be present.

The environmental model showed that oscillations in daily and seasonal temperature and precipitation affect vector are the main risk factors associated with disease incidence. Variations in temperature and precipitation affect vector development and indirectly affect vegetation abundance, which is intrinsically associated with disease. The environmental model explained where the disease is occurring and the socioeconomic model explained, in terms of human population, what can be causing the disease.

Geographic information system, remote sensing and ENMs are powerful tools that can be used to identify areas suitable for disease based on a known distribution. These tools can also improve allocation of resources to better control endemics. Having an efficient allocation of resources is vital because the majority of individuals afflicted by diseases are already living in precarious conditions. ENMs can be used to improve the control programs due to the rapid identification of areas suitable for transmission, thus helping to prevent the occurrence of epidemic outbreaks and the establishment of endemic levels of different illnesses in a diversity of municipalities of Brazil.

One limitation of this study was the use of coarse municipality level data especially from IBGE and PAHO in which socio-economic heterogeneity could not be as well observed as it would by using census tract data for instance. The model might be overfitted due to the nature of tha data but still aggregates useful information to represent the incidence behavior of leishmaniasis in Brazil overall.

3.6. References

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Chapter 4: Environmental Risk Factors of Leishmaniasis in Bahia State, Brazil Using NASA Earth Observation Satellites

4.1. Introduction

Environmental changes and ecological disturbances exert an influence on the emergence and proliferation of zoonotic parasitic diseases by altering the ecological balance and context within which vectors breed, develop, and transmit disease causing agents. Change in vegetation is one of the most disruptive changes affecting parasitic vector populations and changes in the type or amount of vegetation provide changed ecological niches and conditions for proliferation of newly arriving and/or adaptive existing vectors and their parasites (Patz et al., 2000).

Leishmaniasis, an endemic parasitic disease transmitted to humans via the bite of sand flies, remains a severe public health problem in Brazil. The disease burden is in fact increasing in many areas and control programs for leishmaniasis are highly dependent on new research progress to obtain better tools and more cost-effective strategies for case management and vector control (Desjeaux, 2004).

The use of satellite remote sensing technology has shown promise for assessing the risk of vector-borne diseases at different spatial scales (Bavia et al., 2005). Although remote sensing products do not identify the vectors themselves, they can be used to characterize the environment in which the vectors thrive. Earth observing satellite measurements of environmental conditions have distinct advantages over ground measurements because they can be collected repeatedly, automatically, and they are considerably faster to obtain over broad synoptic coverage areas. For the present study, satellite data were used to generate three different vegetation indexes and to extract precipitation and elevation information that was then correlated with incidence of leishmaniasis in Bahia State, Brazil.

Moderate Resolution Imaging Spectroradiometer (MODIS Terra) satellite imagery was collected and used to compute the Normalized Difference Vegetation Index (NDVI), Normalized

Difference Moisture Index (NDMI) and Normalized Difference Water Index (NDWI) data products. Vegetation indices are measurements of the structure of vegetation coverage and physiological parameters associated with photosynthesis (Herbei et al., 2012). As an indicator of vegetation canopy greenness, the NDVI has been found to be an accurate and reliable means of detecting vegetation health, vigor and changes in biomass (Kidwell, 1990).The NDMI is a foliar moisture index that highlights areas of healthy green vegetation with high moisture content and disturbed areas of vegetation with low moisture content (Jin and Sader, 2005). Like the NDMI, the NDWI has been used to detect and monitor the moisture condition of vegetation canopies over large areas (Jackson et al., 2004).

In Latin America, changes in vegetation have been correlated with increases in leishmaniasis incidence. Werneck et al. (2002) were able to correlate vegetated areas with the occurrence of leishmaniasis in Brazil, and Fernandez et al. (2010), generated regression models that showed the influence of percentage of the surface covered by trees and bushes 50 meters away from houses which had reported visceral leishmaniasis. Saraiva et al. (2011), using Google Map images observed that green areas and wooded plots existed near all of the sites studied at distances that were shorter than the flight capacity of vectors.

Risk of cutaneous leishmaniasis (CL) or visceral leishmaniasis (VL) has been associated with proximity to woodlands, elevation, and temperature (Bavia et al., 2005; Chaves and Pascual, 2006; Elnaiem et al., 2003; Feliciangeli et al., 2006; Franke et al., 2002). Valderrama-Ardila et al. (2010), observed that incident cases of leishmaniasis in a Sub-Andean Zone of Colombia were concentrated in a band of medium altitude, between 1,000–2,000 meters. Lysenko (1971) demonstrated that the breeding success of sand flies is more dependent on the duration of rainfall than the intensity. Bhunia et al. (2010), observed that the abundance of *Phlebotomus argentipes* was positively correlated with the rainfall on the Gangetic Plain in India. Annual rainfall appeared to be the most important predictive variable affecting both the probability of presence and the actual incidence of VL in Sudan (Elnaiem et al., 2003). Rainfall

can affect the vector and the reservoir hosts by affecting the vegetation, the diurnal temperature range, and the relative humidity of a given area.

NASA EOS provides a potentially powerful and useful tool to identify favorable environmental conditions for the transmission of leishmaniasis in Bahia, Brazil (Bavia 2005). The capability of identifying and monitoring risk areas using remote sensing allows for the efficient use of available resources to achieve potential prevention of leishmaniasis transmission by providing fast and affordable identification of areas in need for target control practices. Given previous research using remote sensing products to assess environment with disease vectors, there exists the possibility of improving control by considering multiple vegetation indices as well as commonly used climatic/environmental features as NDVI, precipitation and elevation. The objective of this study was to use products from NASA Earth Observing Systems (EOS) to calculate three different indices derived from MODIS imagery, extract precipitation and elevation data, and then evaluate their applicability in the study of leishmaniasis in Bahia, Brazil.

4.2. Material and Methods

4.2.1. Study Area

Bahia, one of 26 states in Brazil, occupies an area of 567,295 square kilometers on the northeastern Atlantic coast of Brazil with a tropical, semi-arid climate and average temperatures between 19.2 to 26.6 °C. Bahia is composed of 417 municipalities and has a population of 14.5 million (Figure 4.1) (IBGE, 2010).

The study period for this project included data at five-year intervals, 2001, 2006, and 2011. All twelve months were included in each study year so that patterns between the wet and dry seasons could be analyzed. The five-year intervals within the study period were chosen, rather than each year, so that climatic/environmental effects, if any, could be observed over the past decade and considered during analysis.

Figure 4.1. Study Area, Bahia State, Brazil.

4.2.2. Data Acquisition

Leishmaniasis data was provided by the State Secretary of Health and Surveillance (SESAB) in Brazil (Bahia, 2012). The data included leishmaniasis cases per municipality per month for every year within the study period.

The MODIS sensor onboard the Terra satellite, MOD09A1 data product was downloaded from the USGS Global Visualization Viewer (GLOVIS) for this study. The MOD09A1 is a level 3, 8-day product which is comprised of seven visible, near infrared, short-wave infrared, and mid-infrared bands. The spatial resolution of the imagery used was 250 meters (MODIS, 2010). MOD09A1 images covering the entire state of Bahia were collected each month for the three years analyzed in this study. Images with less 20% of cloud coverage

were selected per month and an annual average composite was derived from the monthly images. The bands 1, 2, 5 and 6 were used for calculating the indices.

The Tropical Rainfall Measuring Mission (TRMM) product 3B43 used for this study uses a combination of the monthly multi-satellite product and gauge data to create the monthly estimated rainfall amount (Huffman, 2007). The TRMM 3B43 version 7 data was obtained through Goddard Space Flight Center's Giovanni (GES-DISC Interactive Online Visualization and Analysis Infrastructure) TOVAS (TRMM Online Visualization and Analysis System) (Liu et al. 2012). The annual composite was prepared the same as for the indices.

Shuttle Radar Topography Mission (SRTM) data was downloaded from DIVA-GIS as a digital elevation model product for Brazil (DIVA-GIS, 2012).

4.2.3. Data Processing

The data provided by SESAB was recorded separately for CL and VL according to incidence of leishmaniasis cases per month per municipality and the annual records for the diseases was calculated. Population data from the Instituto Brasileiro de Geografia e Estatística (IBGE) for each municipality was used with the health data to calculate the incidence of disease per 10,000 inhabitants.

Four MODIS scenes were necessary to cover the study area. The images were acquired and a mosaic was created in order to produce a single image covering the state for each month. The images used in each scene were from the same collection time period (i.e., same week). After the monthly mosaics per year were developed, the three indices were calculated (Figure 4.2).

The three indices calculated were:

1) Normalized Difference Vegetation Index (NDVI): using the near infrared band and the visible red band to determine changes in greenness on the earth's surface based

on the density of green chlorophyll in vegetation (Voiland 2009). NDVI is calculated according to the equation:

$$NDVI = {(NIR - Red)}/{(NIR + Red)}$$

Equation 1. Normalized Difference Vegetation Index (NDMI) (Voiland, 2009).

Where NIR = 0.86µm (MODIS band 2) and Red = 0.66µm (MODIS band 1) (Hayes et al. 2008);

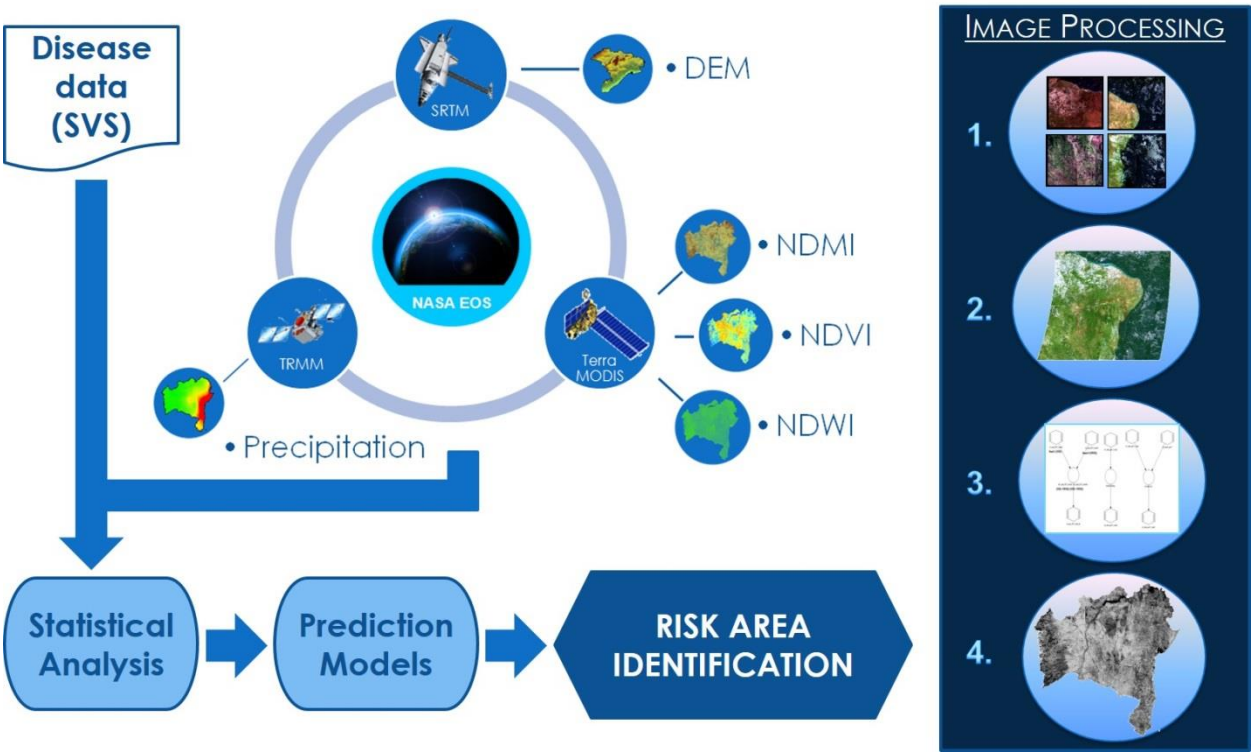

Figure 4.2. Schematic of Data Processing and Analysis.

2) Normalized Difference Moisture Index (NDMI): a moisture index that measures the amount of moisture by sensing characteristic differences between the very reflective near infrared band and the water absorbed short-wavelength infrared band. This index highlights areas of healthy green vegetation with high moisture content and disturbed areas of vegetation with low moisture content using values also ranging from +1 and -1 (Jin 2005). NDMI was calculated as follows:

$$NDMI = {(NIR - SWIR)}/{(NIR + SWIR)}$$

Equation 2. Normalized Difference Moisture Index (NDMI) (Jin, 2005).

Where NIR = 0.86μm (MODIS band 2) and SWIR = 1.64μm (MODIS band 6).

3) The Normalized Difference Wetness Index (NDWI) detects liquid water content in vegetation. Using the near-infrared band and a particular short-wave infrared band, NDWI measures vegetative liquid water in the canopy. NDWI was calculated using the equation:

$$NDWI = {(NIR - MIR)}/{(NIR + MIR)}$$

Equation 3. Normalized Difference Water Index (NDWI) proposed by Gao (1996).

Where NIR = 0.86μm (MODIS band 2) and MIR = 1.24μm (MODIS band 5).

TRMM accumulated rainfall totals were derived for each month during the years 2001, 2006, and 2011, for an area encompassing the state of Bahia in Brazil. The data was interpolated using an ordinary exponential kriging technique in ArcGIS to create a raster file displaying the rainfall distribution across the study area. The data for each of the three vegetation indexes, TRMM and SRTM was extracted by polygon for each municipality in Bahia state according to month and year of occurrence of leishmaniasis.

4.2.4. Data Analysis

Pearson's correlation and logistic regression were used to analyze the variables that most influenced the occurrence of VL and CL in the state. Analysis of Variance (ANOVA) was used to identify significant differences over time. Models for prediction of VL and CL in Bahia State were performed using the Chi-Square analysis and Fisher's exact test. The accuracy of the prediction models were assessed using the area under the receiver operating characteristic

curve (ROC). Statistical analyses were performed using SPSS© version 18.0 (SPSS Inc., Chicago, 2009) and SAS© version 9.1.3 (SAS Institute Inc., Cary, 2004).

4.3. Results

In the years 2001, 2006 and 2011, SESAB recorded a total of 12,456 cases of leishmaniasis of which 10,782 were for the cutaneous form of this disease and 1,674 for the visceral form. VL was reported in approximately 59% of the municipalities in Bahia state, while CL was present in approximately 70% of the municipalities. The spatial distribution of VL and CL is shown in Figure 4.3.

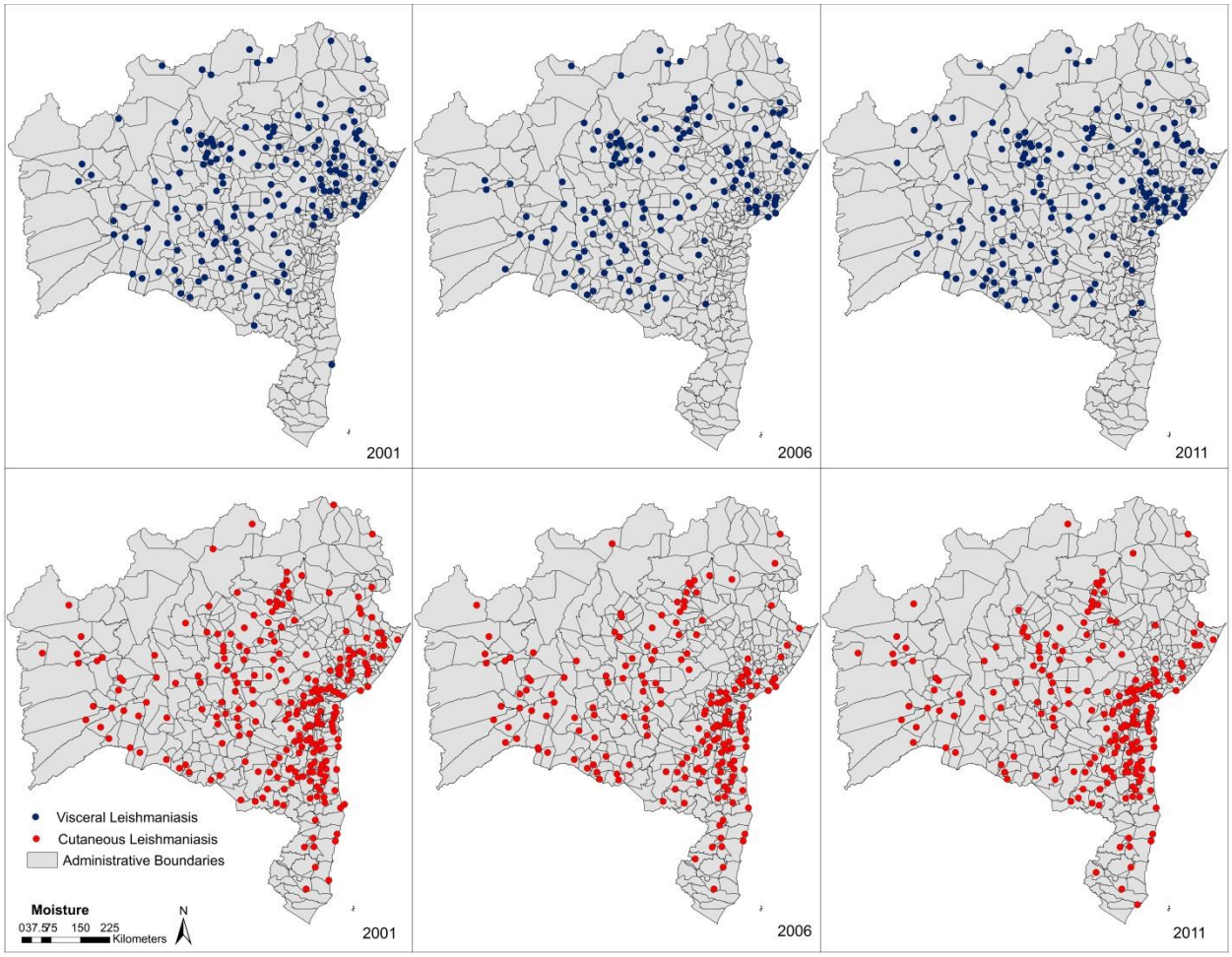

Figure 4.3. Spatial Distribution of CL and VL per Year in Bahia State, Brazil - Top row, cases of CL recorded in the state during the years 2001, 2006 and 2006. Bottom row, cases of VL recorded in the state during the years 2001, 2006 and 2011 (SINAN/SVS/MS, 2012).

The visceral form of disease is concentrated in the north and central region of the state while the cutaneous form was mainly concentrated along the coast and only sparsely occurred in the southern part of the state.

A significant difference between the number of cases reported each year (p= 0.0201) is shown in Figure 4.4A. It was also observed that the peak in cases of both CL and VL occurred in January, February and March, in all three years observed, with a peak number of cases occurring in January (p=0. 0158) (Figure 4.4B).

The mean NDMI observed in the study period was 0.084 (SEM±0.004). For NDVI the mean observed was 0.561 (SEM±0.004) and for NDWI the mean of -0.064 (SEM±0.001). No significant oscillation between months and years in the vegetation indices values analyzed was observed, which suggests the potential of transmission throughout the year (Figure 4.5).

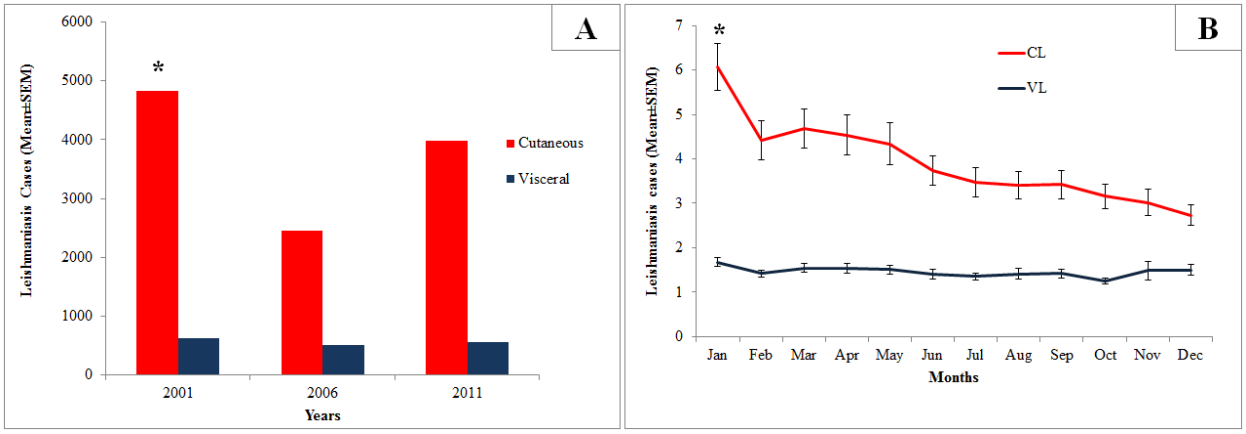

Figure 4.4. Changes in Leishmaniasis Cases Reported in Bahia State Overtime - Total number of cases and mean number of cases (±Standard Error of the Mean (SEM)) by year (A) and by month (B) are shown. Asterisks represent significant differences at the 0.05 confidence level.

Pearson's correlation analysis indicated that both forms of disease tend to increase when environmental factors of moisture (NDMI r=0.23; p<0.001) (Figure 4.6), vegetation (NDVI r=0.17; p<0.001) (Figure 4.7), water content of plants in the canopy (NDWI r=0.25; p<0.001) (Figure 4.8) and precipitation (r=0.21; p<0.001) (Figure 4.9) were at their highest. June and December were the wettest months in all three years studied, with the highest precipitation observed between 200 and 276 mm/month ($p < 0.05$).

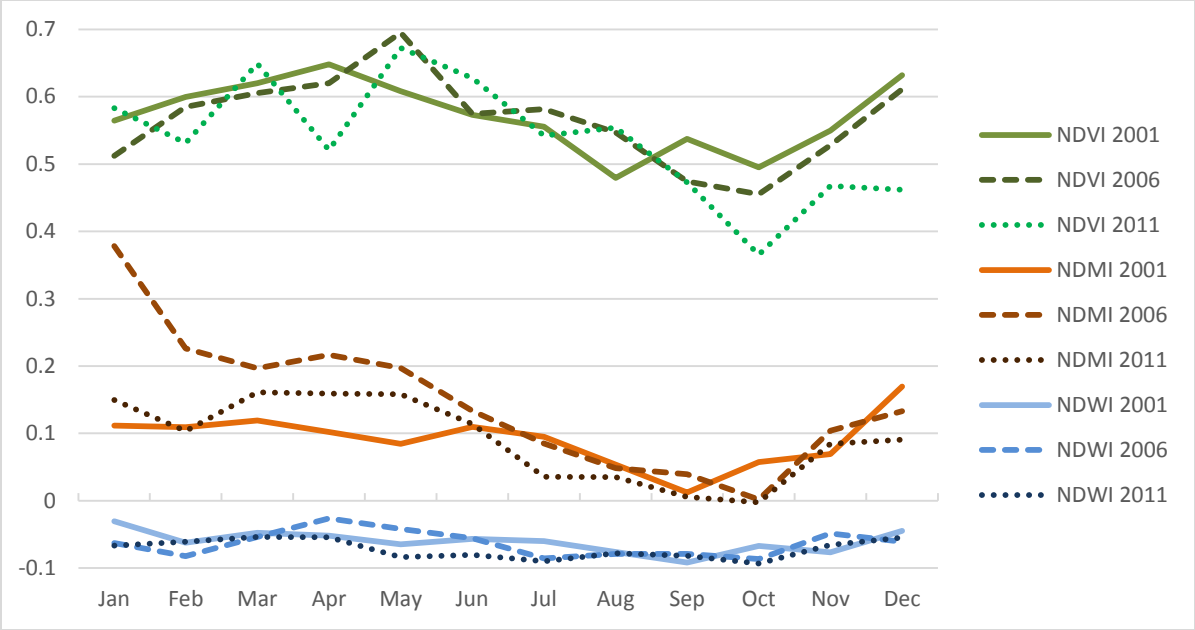

Figure 4.5. Seasonal Dynamics of NDMI, NDVI and NDWI Between Months. Mean vegetation indices values (±SEM) for each year and month are shown.

An inverse relationship of elevation and VL and CL cases was observed, with leishmaniasis decreasing as elevation increased. This tendency follows the same trend as the human population, which tends to be highest in low lying areas (Figure 4.10).

Models developed for prediction of leishmaniasis in Bahia State showed that the occurrence of both forms of leishmaniasis may be influenced by NDMI, NDVI, NDWI and precipitation (R^2= 0.370; p<0.001). The likelihood of having either form of disease (CL or VL) is the same when elevation is considered. In areas with high precipitation, CL is 3.6 times more likely to occur than VL. When considering vegetative moisture (NDMI), CL is 2.11 times more likely to occur than VL. The odds of CL occurrence increase 5.5 times when vegetation (NDVI) is considered and increase 13.5 times when liquid water content of vegetation canopies (NDWI) is considered.

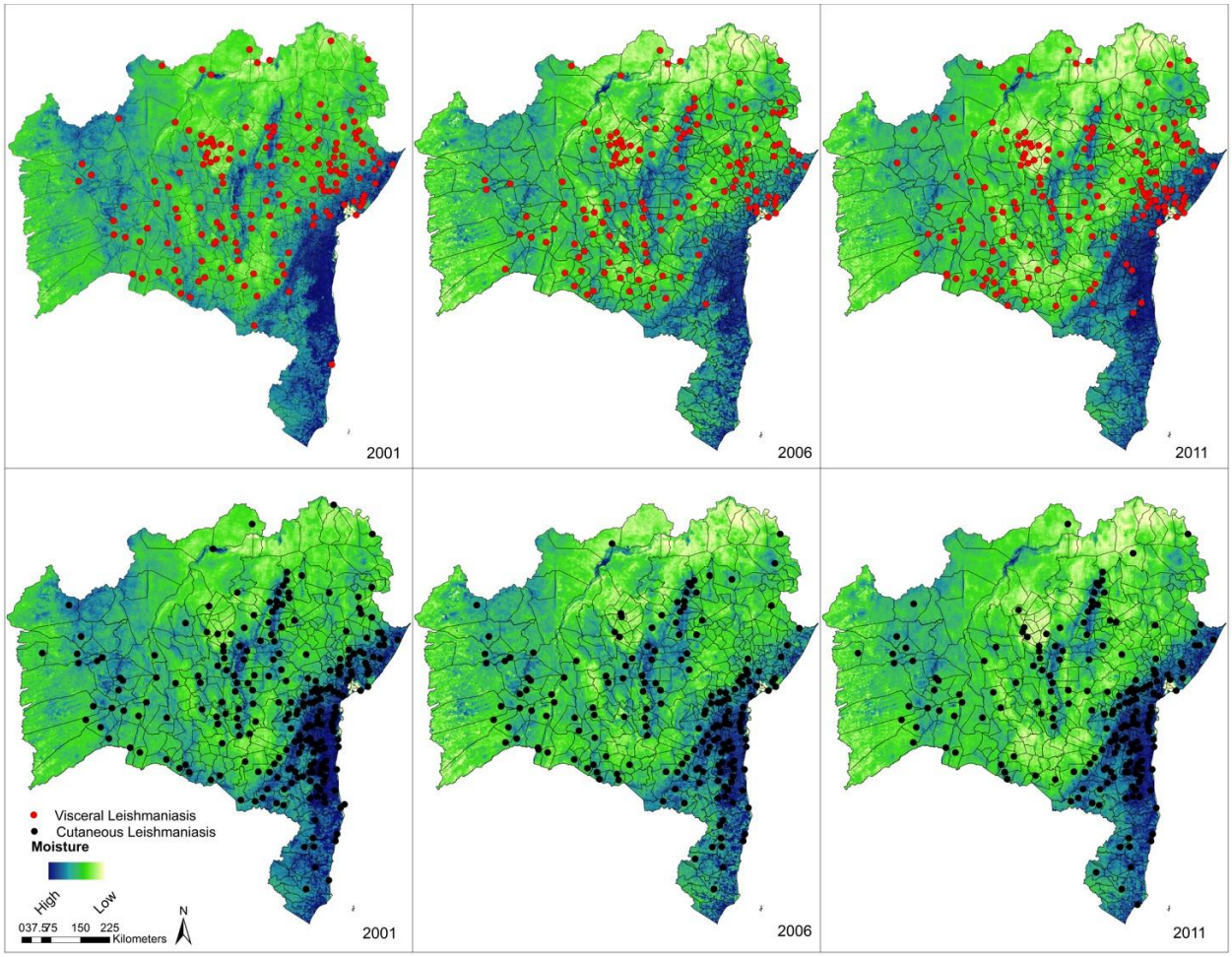

Figure 4.6. NDMI Annual Average Map - The map shows areas ranging from highest to lowest values (1 to -1) derived from MODIS imagery. Each map represents an annual composite for vegetation moisture in Bahia state and disease incidence data for each year in the study period.

Areas at risk of CL and VL were then mapped using the probability of having either form of disease, considering elevation, precipitation, moisture (NDMI), vegetation (NDVI) and water content of vegetation (NDWI). It was observed that for the cutaneous form of disease, coastal areas are at higher risk but there was also high at a specific area on the northwest region of the state.

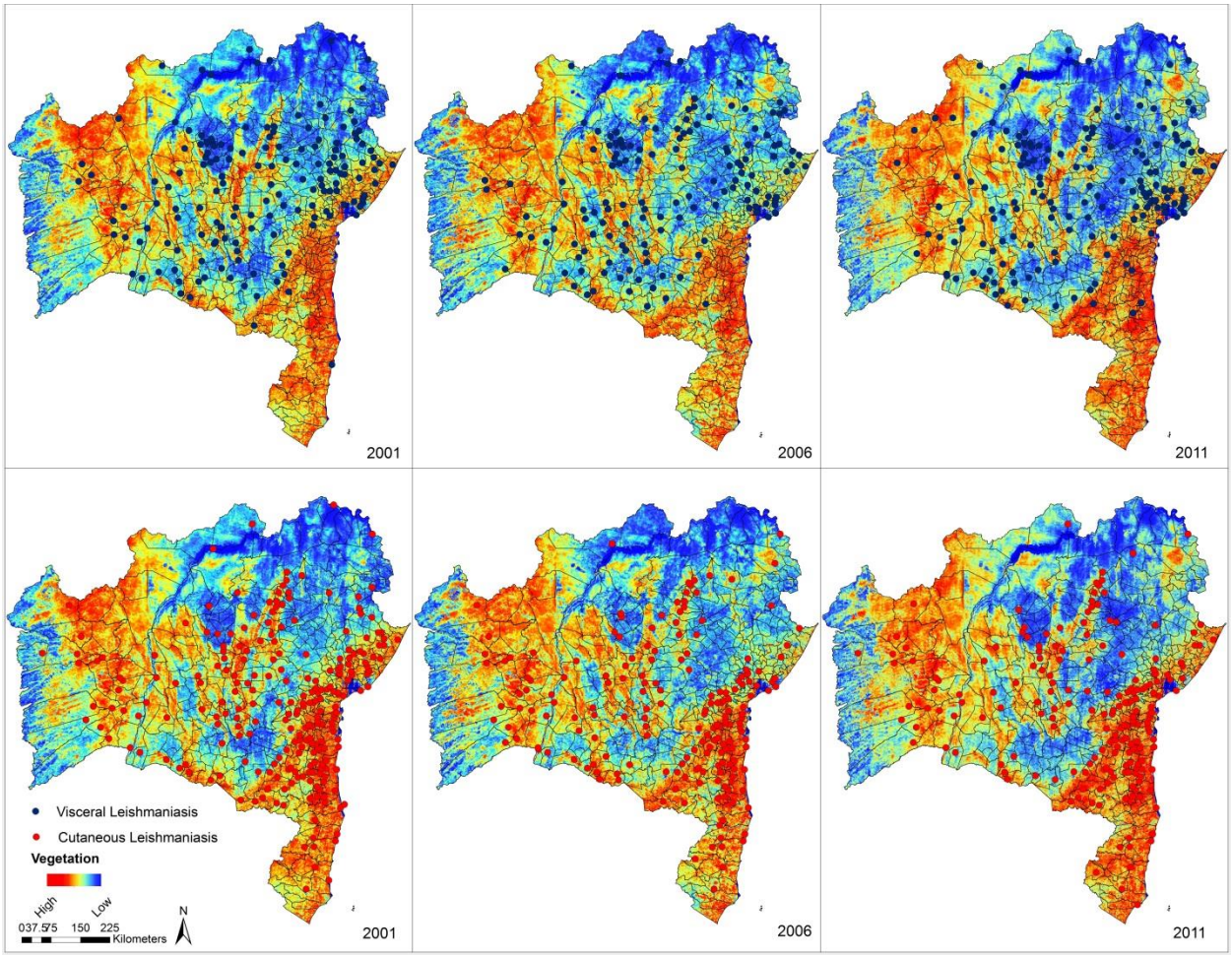

Figure 4.7. NDVI Annual Average Map - The map shows areas ranging from highest to lowest values (1 to -1) derived from MODIS imagery. Each map represents an annual composite for vegetation in Bahia state and disease incidence data for each year in the study period.

For the visceral form of the disease, areas at higher risk included the north and southwest regions of the state. Although specific areas for occurrence of one or the other form of the disease was identified, it was also possible to note areas where both forms of leishmaniasis overlap and even within a municipality, the risk levels differ, which provide useful information for control programs about where control action should be prioritized. The accuracy of the prediction models were assessed using the area under the receiver operating characteristic curve (AUC=0.72) (Figure 4.11).

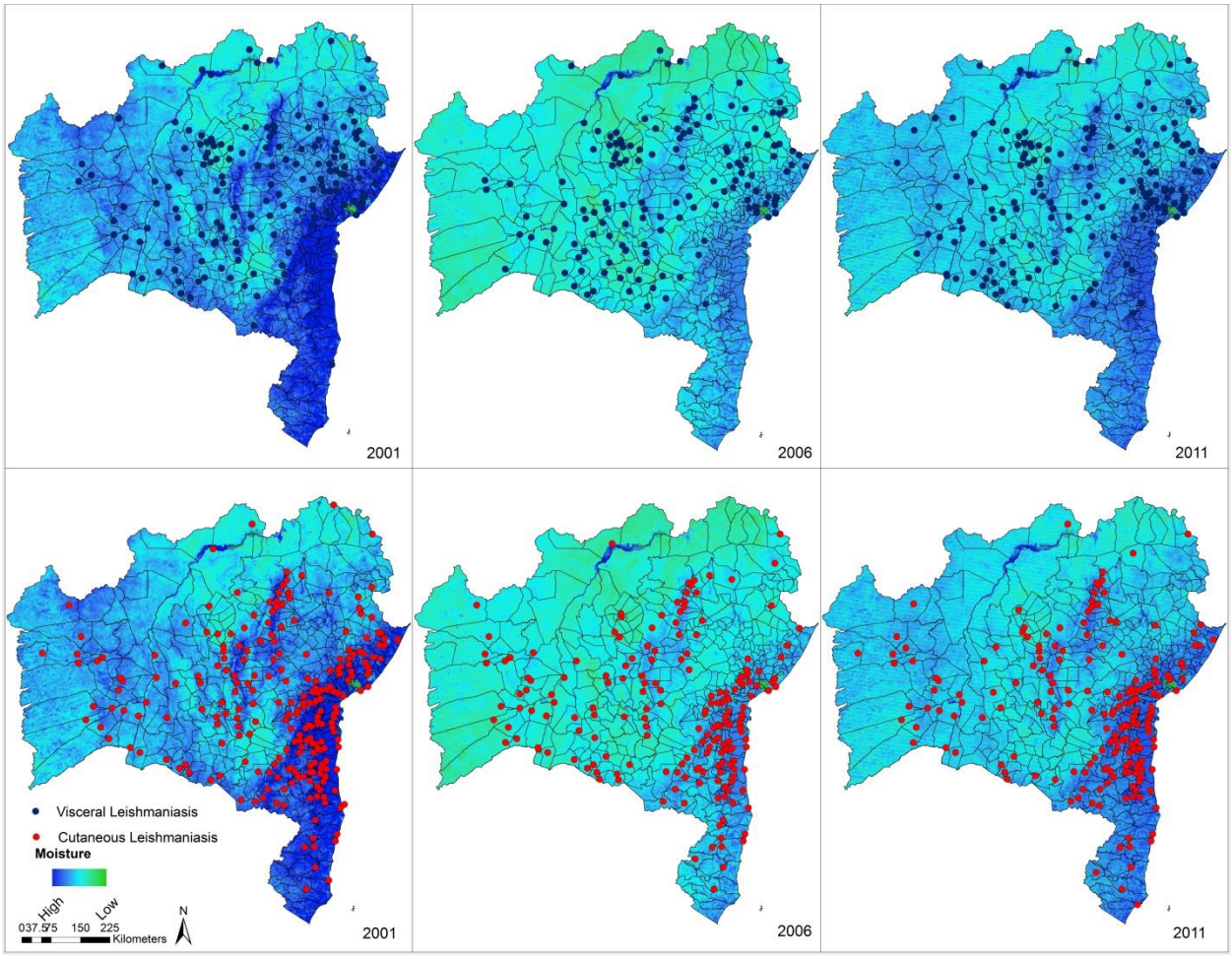

Figure 4.8. NDWI Annual Average Map - The map shows areas ranging from highest to lowest values (1 to -1) derived from MODIS imagery. Each map represents an annual composite for water contents of vegetation in Bahia state and disease incidence data for each year in the study period.

4.4. Discussion

NASA EOS provides a potentially powerful and useful tool to identify favorable environmental conditions for the transmission of leishmaniasis in Bahia, Brazil (Bavia 2005). The capability of using remote sensing to identify, map and monitor areas favorable for transmission of CL and VL may allow risk of disease to be modeled and analyzed in control programs 'on demand' for prevention of leishmaniasis transmission.

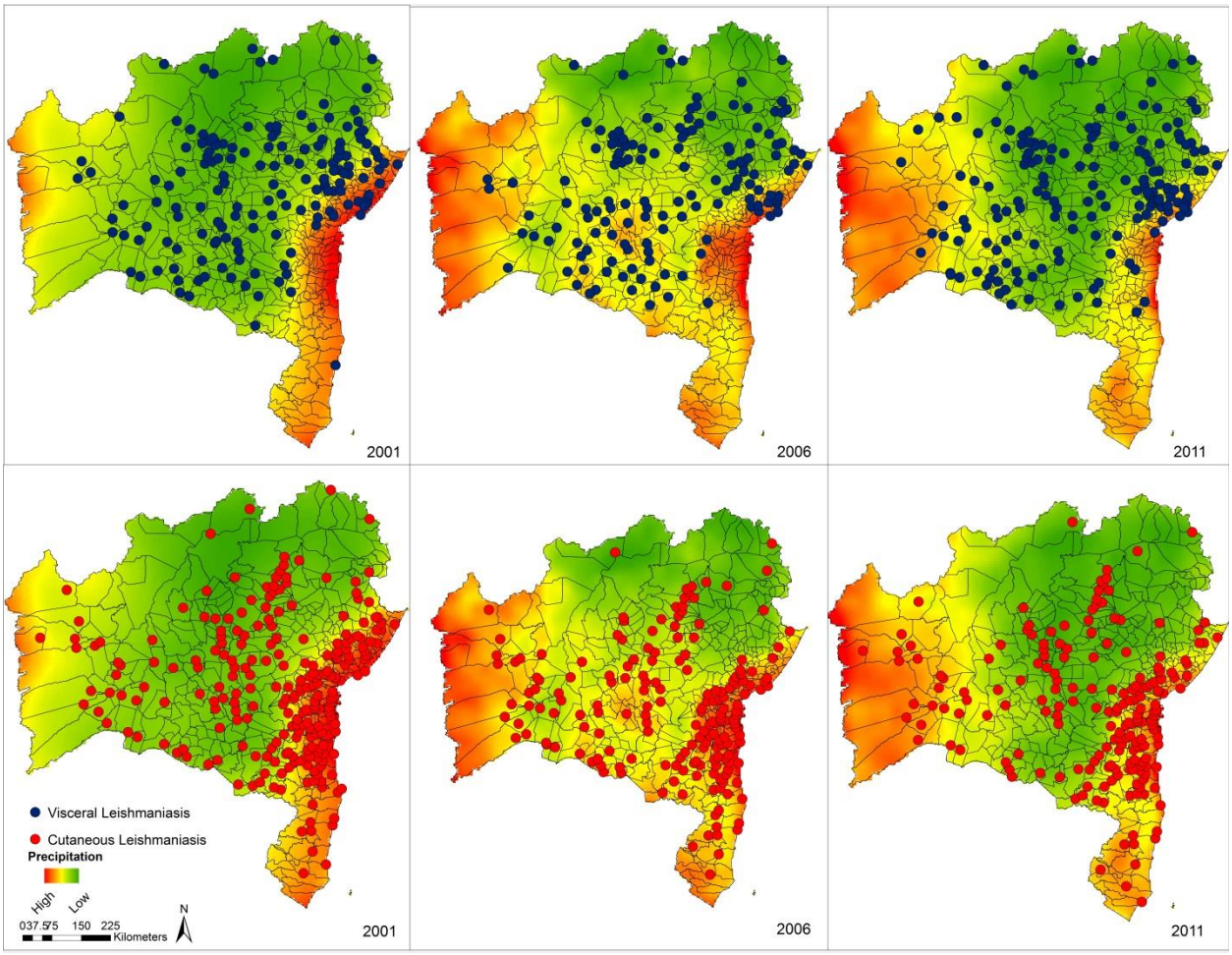

Figure 4.9. Annual Precipitation Derived from TRMM - Maps represent rainfall accumulation and incidence of VL or CL for each year in the study period.

The objective of the study reported here was to use products from NASA Earth Observing Systems (EOS) to calculate three different vegetation indexes, extract precipitation data from TRMM satellite data and elevation data from SRTM, and then evaluate their applicability in the study of leishmaniasis. Areas at risk of CL and VL were then mapped using the probability of having either form of disease, considering elevation, precipitation, moisture (NDMI), vegetation (NDVI) and water content of vegetation (NDWI) as surrogates of climatic conditions.

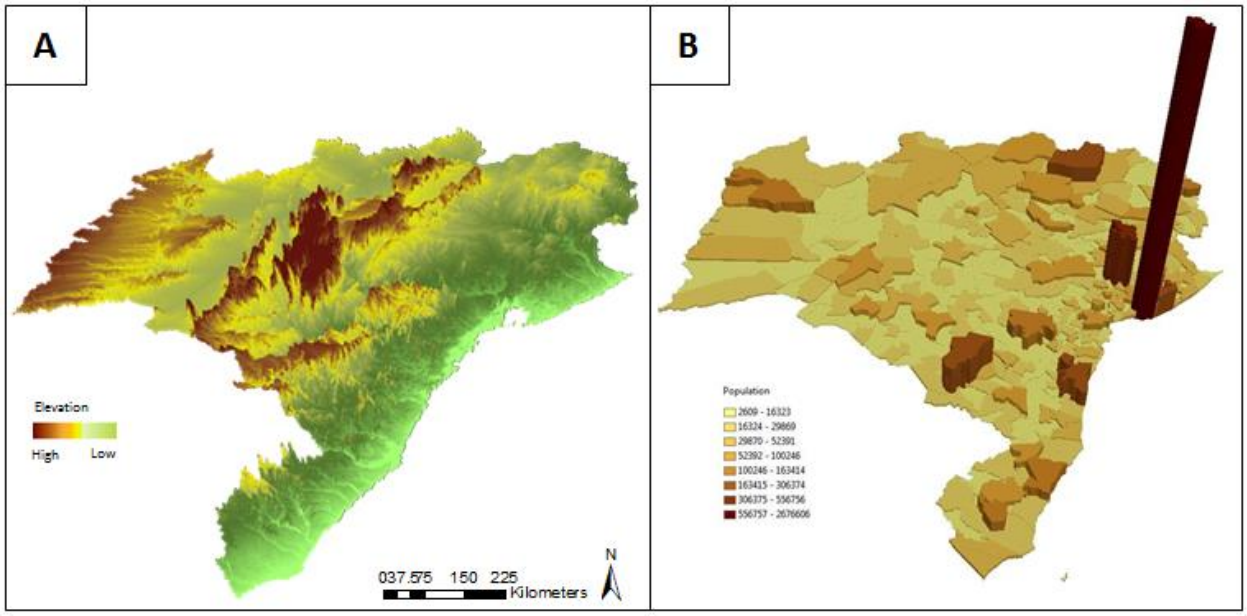

Figure 4.10. 3D Maps of Elevation Data Derived from SRTM (A) and Population Data from the IBGE Population Census of 2010 (B).

4.4.1. Leishmaniasis Distribution and Seasonality

The introduction of environmental features in epidemiological models is essential since they dictate parasite and vector development (Correia et al., 2005; Dias et al., 2007). The climate in Bahia state is tropical with stable and elevated temperatures throughout the year and two distinct wet seasons. A tropical climate is predominant in the coastal areas, while in the interior areas, which occupy most of the land, the climate is predominantly semi-arid. Along the coast line there are areas where annual rainfall exceeds 55 mm. The wet season for the coastal area is from April to July, but rainfall is observed throughout the year. In the semi-arid region the annual rainfall never exceeds 24 mm. The dry season is from April to October, but even during the wet season, rainfall is scarce. In the semi-arid northeast region, where the majority of records of both CL and VL were reported, dry and rainy seasons are clearly defined and there is evidence that the population density of the sand fly vector is low during the dry season and increases after the end of the rainy season (December–April), reaching its highest density level around May. These observations support reports of peak numbers of cases in the months of

January, February and March when an increase in vector density favors transmission, thus the increasing incidence after this period (Deane and Deane, 1962; Franke et al., 2002b; Sherlock, 1996).

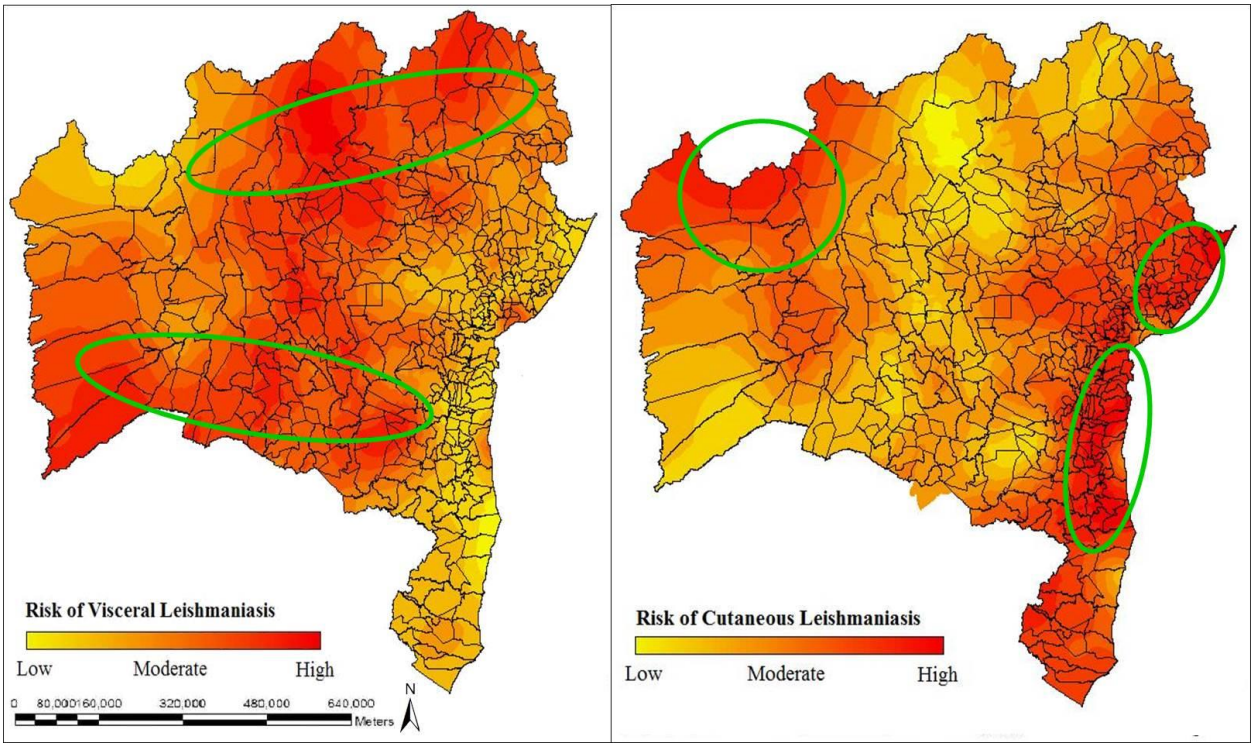

Figure 4.11. Risk of Leishmaniasis in Bahia State Derived from the Calculated Probability of Incidence of CL or VL Based on Environmental Features - Probability was calculated by polygon and a raster surface was created.

Bahia is one of the Brazilian states most affected by both the cutaneous and visceral forms of leishmaniasis. For CL, the geographic distribution in the state is restricted mainly to tropical and temperate regions that provide habitats suited for the sand fly vector species that transmit CL (*Lutzomyia whitmani, Lu. intermedia, Lu, migonei, Lu. flaviscutellata, Lu. fischeri, Lu. Lu. ayrozai, Lu. anduzei, Lu. davisi, Lu. hirsuta hirsuta* (Brasil, 2007)) such as the Amazon Forest and the Atlantic Forest (Fig 4.9). CL is widespread in the state of Bahia, however, with existence of foci of infection in all regions. (Andrade et al., 2010; SUVISA, 2013). For VL, which is transmitted only by *Lu. longipalpis*, areas of occurrence in Bahia are increasing, especially in

the semi-arid region in the north and west regions of the state, reportedly in association with the new implementation of agricultural projects (Franke et al 2002a). Increasing incidence in municipalities in the east region of the state, especially north to the state capital Salvador, has been attributed to a progressive increase in human population density (Oliveira and Araújo, 2003; Brasil, 2014; Franke et al. 2002). It has been reported that Bahia had more municipalities with reported cases of CL than VL and proposed that the emergence of both forms of the disease in the state, especially towards the west region, was a consequence of environmental changes associated with the development of soy bean and rice production (Franke et al., 2002).

4.4.2. Normalized Difference Moisture Index (NDMI)

The NDMI index gives an indication of the wetness of the land surface. It evaluates the different content of humidity from the landscape elements, especially for soil, rocks and vegetation. This index is also a good indicator for dryness (Herbei et al., 2012). High NDMI values indicate the existence of more soil surface moisture and low value indicates low soil surface moisture content. The mean NDMI observed in this study indicates a dry soil, which is observed in the state which the majority of the land represents a semi-arid environment. By contrast, high moisture is observed throughout the coast where a tropical climate predominates (Goodwin et al. 2008; Sahu, 2014). A field survey carried out for collection of soil moisture data by Mallick et al. (2012), found a relationship between soil moisture from field sample collection and NDMI, indicating that this is a useful index to measure moisture from soil, vegetation and even predicts surface temperatures based on NDMI.

Soil moisture has been shown to play a significant role in leishmaniasis prevalence being concurrently linked to vector and host (Bhunia et al., 2010). Wassenberg et al. (2002), observed that sand fly density was positively correlated to soil moisture being higher in the relatively humid plots of Nizzana ruins, Israel and much lower in the drier sandy soil of Mt. Keren. Saraiva et al. (2011), observed that humidity was a favorable factor for the presence of

sand flies in dwellings and that green areas displayed a much higher [specific] abundance of such insects than in exclusively urban environments. Bhunia et al. (2011), found that moisture content in surrounding areas and less dense bodies of water affect sand fly density. An increase in moisture and humidity promoted the multiplication of the sand fly population, which then promoted disease transmission. This provides evidence of the impact of moisture and small water bodies as a source of moisture to the environment in the occurrence of VL. It also corroborates the interrelationship of the climatic/environmental factors that altogether provide ideal conditions for sand flies, thus making the NDMI a suitable index for identification of areas with favorable characteristics for vector development.

4.4.3. Normalized Difference Vegetation Index (NDVI)

NDVI is a vegetation index used to measure vegetation cover and also water bodies over the earth surface and is determined by the chlorophyll absorption in the red wavelengths (Jackson and Huete 1991; Tucker et al. 1985; Tucker and Choudhury 1987). NDVI has been widely used to identify habitats of vectors of diseases such as Rift Valley Fever (Anyamba et al., 2001) and schistosomiasis (Bavia et al., 2001) as well as leishmaniasis (Carneiro et al., 2004; Nieto et al., 2006).

Bavia et al. (2005), reported that NDVI was one of the most important risk factors in the northwest area of Bahia State. In their study, low NDVI values, which represent human made features such as roads and construction materials (i.e. buildings), were related to high numbers of sand flies and high numbers of human and canine cases of the visceral form of leishmaniasis. The mean NDVI value observed in this study showed that there is no dense forest cover where cases of leishmaniasis have been reported, but instead areas of agricultural lands. In Argentina, Quintana et al. (2010), observed higher sand fly density and CL prevalence in areas of modified primary vegetation, associated with deforestation and the subsequent anthropic modifications in the deforested areas.

Carneiro et al., (2004) observed a positive correlation between low NDVI and occurrence of VL in Feira de Santana, Bahia, but in a similar study, Bavia et al., (2011) did not observe a significant relationship between VL and NDVI in Conde, Bahia. However, Cerbino Neto et al., (2009) identified an interaction between population growth and NDVI in urban areas of Teresina in Piaui state. This interaction demonstrates the impact of recent, rapid occupation of the city's peripheral areas on the risk of VL. Population growth in an area that is already urbanized has a much smaller impact on VL incidence than the same level of growth in an area with heavy vegetation cover; NDVI can thus be a useful tool in identifying such pockets of dense or scarce vegetation coverage. Heavy vegetation in endemic areas can provide growth and shelter sites for sand flies.

4.4.4. Normalized Difference Water Index (NDWI)

NDWI is used to identify the interface between land and open water bodies. The NDWI proposed by Gao (1996) estimates water content of vegetation canopy and is referred to as a good indicator of drought conditions. Gu et al. (2008) found that NDWI values exhibited a quicker response to drought conditions than NDVI.

In this study it was observed that the NDWI mean was characteristic of vegetation cover or more strictly, it identified water content of vegetation in the area. This was also the index that increased the chances of having leishmaniasis in the state the highest. Although not very utilized in studying diseases, NDWI can be a useful tool in the public health sphere. McFeeters (2013), provided a step by step use of NDWI to identify swimming pools that could be providing habitat for mosquitoes to breed, and thus be incorporated into mosquito abatement programs. Zhang et al. (2009), have demonstrated the use of NDWI to study the location of active transmission sites of *Schistosoma japonicum* in China and such an approach provided a useful tool in the fields of vector-borne and environment-related diseases. According to Lainson and Rangel (2005), the immature stages of sand flies do not grow in wet locations, so areas of high

humidity in potential habitat areas may have hindered insect development. The sand fly larvae is terrestrial, thus the impact of water is not extreme in sand fly development other than for providing the necessary moisture and humidity for life maintenance.

Since this index is also a good indicator for drought, it is important to note that droughts have been shown to favor the development of epidemics in Latin American countries. Prolonged droughts in semi-arid north-eastern Brazil have provoked rural-urban migration of subsistence farmers, and a re-emergence of visceral leishmaniasis. Such outbreaks could be the result of human migration due to drought, environmental degradation or economic reasons and may lead to the spread of diseases in unexpected ways, and new breeding sites for vectors (Euroclima, 2012). Sand flies are insects with predominantly nocturnal habits and tend to seek shelter in places where there are no major changes in microclimate (Brazil and Brazil, 2003). The vector of VL in Bahia, *Lutzomya longipalpis*, has been often found inside residences during drought periods (Sherlock, 1996). Prolonged drought periods can impact vector density and contribute to a gradual increase in the potential risk of some related epidemiologic factors, such as waning population immunity and increase in the susceptible population in the endemic areas (Franke et al., 2002b).

An interesting overview that could also support the applicability of NDWI in studying leishmaniasis is the effect that water and sap content from vegetation, especially in arid or irrigated areas, on sand fly saliva quality and quantity. Sand fly saliva contains molecules involved in blood feeding and vertebrate host immune response (Coutinho-Abreu and Ramalho-Ortigao, 2011; Schlein and Jacobson, 2002). Identification of areas with adequate water in vegetation can lead to prediction/identification of sand fly breeding sites.

According to Crist and Cicone (1984) and Wilson and Sader (2002), NDWI and NDMI are theoretically similar to each other for detection of spatial variation of surface wetness. However, NDWI performed better than other indices in identifying wetland surface water in

Bihar, India and it was also the index that detected more pixels with water and water features in the Sahara-Sahel transition zone (Ashraf and Nawaz, 2015; Campos et al., 2012).

4.4.5. Tropical Rainfall Measurement Mission (TRMM)

The TRMM precipitation data provides rainfall estimates based on 0.25° x 0.25° grid cells. The value for each grid cell is based on the average rainfall over the grid cell area and is placed in the center of each grid cell.

Rainfall requirements suitable for CL transmission are different from those of VL. For both types of leishmaniasis, precipitation has been identified as an important risk factor. Karagiannis-Voules et al. (2013), observed that annual precipitation, precipitation seasonality, precipitation in warmest quarter was a risk factor for CL transmission in Brazil, while low annual precipitation and none extreme precipitation during the warmest quarter was a risk factor for VL. A spatial analysis including environmental variables in Iran found that precipitation was positively associated with CL incidence and that there was a negative association of precipitation with VL (Ali-Akbarpour et al., 2012). A study in northeast Brazil showed an inverse relation of a 3-year mean precipitation and VL incidence (Thompson et al., 2002). In the Yucatan Peninsula in Mexico, a seasonal transmission of CL right after the wet season was observed (Andrade-Narvaez et al., 2003).

As for the vectors of leishmaniasis and precipitation, a study of the vectors associated with CL in Argentina observed that annual precipitation did not contribute to their prediction models (Quintana et al., 2013). *Phlebotomus spp*. in Sudan seemed to be restricted to areas with rainfall between 400-1200 mm (Elnaiem et al., 1998; Thompson et al., 1999). In a northeast state of Brazil, monthly rainfall did not show any influence in sand fly population density, but a clear difference in sand fly capture between wet and dry season in the same areas was observed (Amóra et al., 2010). Precipitation seems to play an indirect role in the incidence of leishmaniasis as well as providing suitable habitat for vector development.

4.4.6. Shuttle Radar Topography Mission (SRTM)

Topographic influence, in special altitude, is of particular importance in predicting disease risk. Elevation by itself may not represent a crucial factor in the incidence of leishmaniasis, but might influence other factors such as distance to water bodies, low vegetation, soil rich in organic matter, which are basic requirements for development and maintenance of the sand fly vectors. Bhunia et al. (2010), from their DEM results observed that the altitude has a relatively strong influence on the distribution of *Phlebotomus* vector in India as the Kala-azar incidence is concentrated at low altitudes with fewer cases in the highlands. These results indicate that a higher incidence should be expected at low elevation as was found to be the case in Bahia state. Population density seems to be an important factor to the distribution of disease, since its decreasing frequency in samples from increasingly higher altitudes, coincides with the distribution of VL cases in India (Bhunia et al., 2010).

The results of the current study in Bahia state agreed with what was observed by Bavia et al., (2011) in which an inverse relationship between leishmaniasis and altitude was observed. Cases of leishmaniasis, both CL and VL, decreased as elevation increased and it seems to follow the same trend as human population, which tends to be highest in low lying areas. Elnaiem et al. (2003), observed that although the elevation did not correlate with VL incidence in Sudan in their preliminary analysis, it appeared as an important variable when used in multivariate analysis indicating that in the final analysis, elevation integrates the effects of many other factors, including distance from rivers. Such results are in agreement with other authors regarding the requirements for environmental preferences of vectors of *Leishmania* parasites (Kawa and Sabroza, 2002; Brasil, 2006). But it is necessary to take into consideration the differences between different geographic areas as well as sand fly species.

The seasonality of transmission of either form of leishmaniasis is characterized not only by the absence of a transmission cycle during a specific season, but also by the lack of potential transmission during the rest of the year (Andrade-Narvaez et al., 2003). The environmental

conditions in Bahia state tend to stay constant with very small fluctuations throughout the year, which reinforces the ability of disease transmission to be continuous, but also in a well-defined environment. It is important to note however that the oscillation in the records of leishmaniasis in relation to environmental factors could also be due to employment of control measures with concomitant intensification of epidemiological surveillance (Bavia et al., 2011).

The different approaches used in this study, could be useful in detecting environmental factors that can be associated with the parasite, vector or reservoir/host and their relationship with leishmaniasis occurrence. They can further provide useful information about the ideal conditions for vector breeding, establishment and dispersion.

4.5. Conclusion

Results of this study indicates that the use of earth observing satellite data products to study leishmaniasis can be an effective tool in identifying the environmental features that influence the disease's occurrence in Bahia State. The environmental features associated with leishmaniasis are different depending on the form of disease (cutaneous or visceral) spatially and temporally.

Precipitation and the vegetation, moisture and water indices used showed the areas that could be more suitable for disease incidence. Vegetation is essential for maintaining vector development as it provides carbohidrates which are the main energy source for both male and female sand flies. Moisture and water surface provide the humidity necessary for the vectors to develop. The sand fly larvae is terrestrial and feed on organic matter so maintaining the humid conditions of the habitat will keep the life cycle of the vector continuing.

To know the timing of the transmission cycle and associated environmental features is important to identify high-risk seasons so that intervention measures such as insecticide spraying can be directed to areas at risk. The cutaneous form of leishmaniasis was more sensitive to environmental factors and NDWI was the index that best depicted occurrence of

both forms of leishmaniasis in Bahia. Vegetation indexes such as NDMI and NDWI provide useful environmental characterization of an area but haven't been used in studying endemic diseases very often.

In future, the results of this study can be refined by utilizing higher resolution satellite data such as Landsat imagery to identify environmental factors on a smaller scale (i.e. cities, villages) and classify the study area based on land use and land cover. Further studies applying the NDMI and NDWI are recommended in order to determine the optimal use of these indices in health applications.

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Chapter 5: Ecological Niche Models for Species of Sand Flies and their Association with Human Visceral Leishmaniasis in Bahia State, Brazil

5.1. Introduction

Sand flies are known to act as vectors for disease microrganisms including protozoans, viruses and bacteria (Shaw et al., 2009). These insects belong to the order Díptera, family Psychodidae. Most psychodids are harmless to humans, but those in the subfamily Phlebotominae are blood sucking, and of most medical entomological importance, mainly as the vector of leishmaniasis (Borror et al., 1992). Leishmaniasis is caused by protozoan parasites from over 20 *Leishmania* species and is transmitted to humans by the bite of infected female phlebotomine sand flies. Over 90 sand fly species are known to transmit *Leishmania* parasites in the Old and New World. In Brazil, about 229 sand fly species have been identified, with 19 species being incriminated as disseminating *Leishmania* to man and animals (Santos et al., 1998; Young and Duncan, 1994; Rangel and Laison, 2003). Leishmaniasis is amongst the six endemic diseases considered by the World Health Organization, as a priority for control worldwide. It is estimated that 1.3 million new cases and 20,000 to 30,000 deaths occur annually. Three forms of leishmaniases have been described: cutaneous (most common form); muco-cutaneous and visceral (also known as kala-azar and the most serious form of the disease) (WHO, 2015).

Visceral leishmaniasis (VL) is a serious public health issue that can lead to death if not properly treated (Brasil, 2014). This disease is considered endemic in Brazil and was previously described mainly in rural zones with semi-arid climate; however, environmental factors such as climate and vegetation no longer represent a barrier to the expansion of this disease (Deane and Deane, 1962; Rebêlo, 1999; Werneck et al., 2008). Vector adaptation to different habitats is probably the reason behind an increase in the incidence of VL as well as its shift from rural settings to urban centers of larger cities and to areas previously described as free of VL (Aguiar and Medeiros, 2003; Barbosa et al, 2006; Deane and Deane, 1955; SINAN/SVS, 2009). Two

species are involved in the transmission of VL in Brazil, *Lutzomyia longipalpis* and *Lutzomyia cruzi*, the latter only occurring in the central west region, Mato Grosso do Sul state (Brasil, 2014).

Factors affecting the spatial positions of vectors and the probability of encountering hosts are important in understanding disease dynamics. Drastic environmental changes caused by migration, socioeconomic factors, urban growth, domestication of the transmission cycle and the insertion of farms and settlements in forest areas can affect the incidence of VL. The impact of climate in human and vector populations have also been reported as affecting leishmaniasis incidence by changing the ecology of some species of sand flies, consequently changing the eco-epidemiology of VL (Brasil, 2014; Franke et al., 2002; Werneck, 2008). Despite major control programs that are in place in Brazil, they have not yet achieved successful results. Vector control was previously focused on the spraying of insecticides in areas where human cases have been confirmed. Nowadays, it focuses on knowledge of the presence, distribution and monitoring of vector dispersion (Brasil, 2014). There remains a need for better control plans for this endemic disease, especially concerning vector abatement.

The use of satellite remote sensing technology has shown promising results in assessing the risk of various vector-borne diseases at different spatial scales (Bavia 2005). Although remote sensing products do not identify the vectors themselves, they can be used to characterize the environment in which the vectors thrive. Medical mapping and modelling tools, using geographic information systems, and remote sensing technology have become a normal part of planning and implementation of disease control programs (Malone and Bergquist, 2012).

Ecological niche modeling (ENM) is another tool that has been used for discovering new habitats or identifying suitable areas based on the occurrence of a species. Such models can provide information that could be useful for decision making personnel in planning or creating surveillance programs and mosquito abatement regimens. Ecological niche modeling uses presence record data in conjunction with environmental data to develop models of habitat range

for a given organism (Peterson, 2001). Studies on the distribution of *Leishmania* and *Lutzomyia* species in Latin America have used ENMs (Nieto et al., 2006; Peterson and Shaw, 2003). Some studies have proposed the use of maximum entropy method (Maxent), to develop such models (Almeida et al., 2013; Gonzalez et al., 2010; Sanchez et al., 2015). Maxent is a machine-learning method that uses the principle of maximum entropy on presence-only data to estimate a set of functions that relate environmental variables and habitat suitability in order to approximate the species' niche and potential geographic distribution (Phillips et al. 2006).

Studies of the distribution of sand flies and leishmaniasis can aid in the development of monitoring and disease control programs in Bahia state. Since information regarding the vector is still scarce, the present study aimed to use ENM to analyze the spatial distribution for VL in humans in relation to sand fly species present, focusing specifically on *Lu. longipalpis* in Bahia state, Brazil.

5.2. Material and Methods

Bahia state is located on the northeastern Atlantic coast of Brazil, latitude 19°57'00"S and longitude 44°31'00"W. The state has an area of 567,295 Km^2, is composed by 417 municipalities and has a population of 14.5 million inhabitants (IBGE, 2010). The climate in the state goes from tropical in coastal areas to semi-arid inland and annual average temperatures vary between 19.2° and 26.6°C. Rainfall ranges from 360 mm to 2,000 mm. The dominant vegetation is tropical forest, mangrove, cerrado and caatinga (Bavia, 1996).

The presence information for the models developed in this study was derived from the Brazil's national information system for notifiable diseases (SINAN). Human records of VL are reported by municipality and the geographic coordinates for each locality was obtained through the Brazilian Institute of Geography and Statistics (IBGE, 2010).

Sand fly presence information was based on entomological monitoring surveys carried out by the Entomology Sector of the Central Laboratory (LACEN) over two years. The monitoring

data includes records of captures of insects from July 2009 to December 2012. Samples were collected using CDC light traps that were installed monthly during four consecutive nights, from dusk to dawn (18:00-06:00) in domestic animal shelters and peridomiciles, according to the guidelines of the Ministry of Health (Brasil, 2010). Sand fly species were identified by LACEN using the identification key proposed by Galati (2003). A total of 66 municipalities (15.83% of the municipalities in the state) were included in the monitoring survey, either as part of the entomological activities already in place at the local health offices or as part of monitoring activities of the entomological surveillance program carried out by LACEN for a period of 2 years, in which 11 municipalities were selected due to their moderate to intense transmission of VL classification. In these municipalities, a total of 141 localities, either urban, rural or village had CDC light traps installed for insect capture. The number of traps installed was defined according to the number of VL cases in the region (Figure 5.1).

The Moderate Resolution Imaging Spectroradiometer (MODIS) product was downloaded from the USGS Global Visualization Viewer (GLOVIS). Vegetation information for the state was then extracted using the vegetation indices: Enhanced Vegetation Index (EVI); Normalized Difference Vegetation Index (NDVI), Normalized Difference Moisture Index (NDMI) and Normalized Difference Water Index (NDWI). Land Surface Temperature (LST day and night) information was also extracted from MODIS images. The methodology for generating the vegetation indices followed the same procedure previously described in Chapter 4. LST and EVI are ready to use products from Terra MODIS (MOD11 and MOD13 respectively) available for download at GLOVIS (http://glovis.usgs.gov/).

A set of 19 bioclimatic variables derived from long-term normal monthly climate data on temperature and rainfall (Bioclim 1-19, http://www.worldclim.org/bioclim, last accessed October 19, 2010) was also used to generate the distribution models. A list of the Bioclim variables is presented on table 5.1.

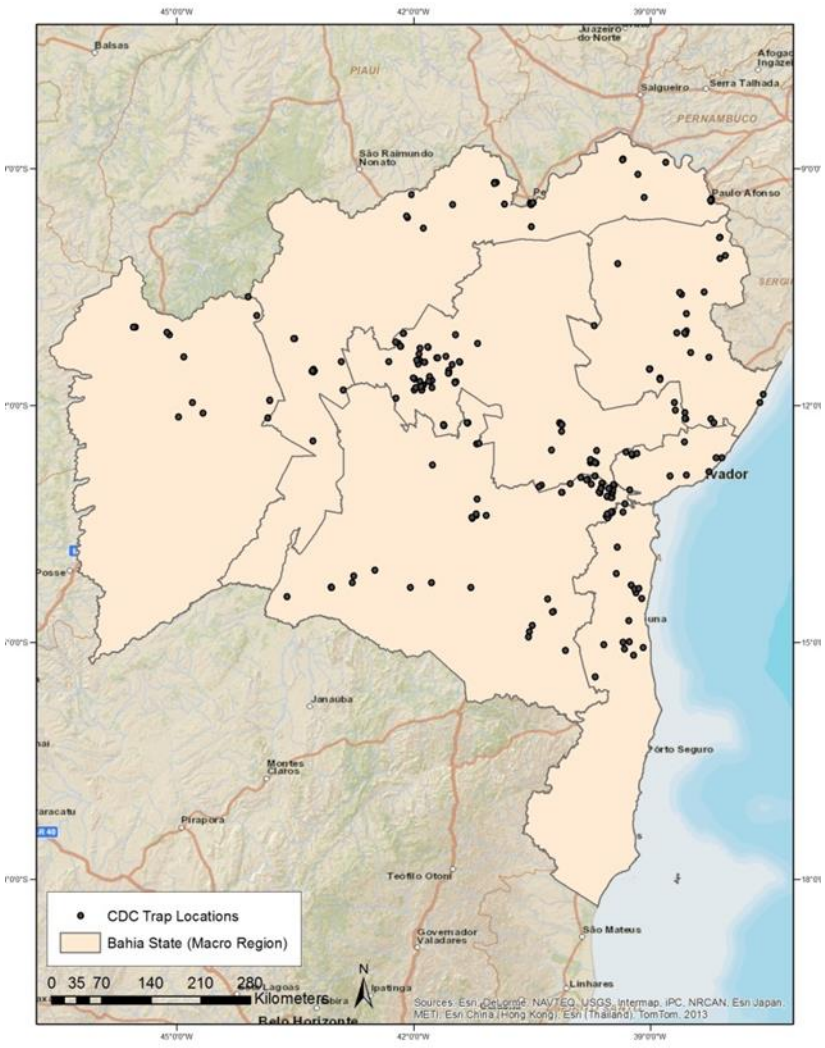


Figure 5.1. Location of the CDC Light Traps from the Monitoring Survey Carried Out by LACEN.

All variables were reduced to a grid resolution of 30 arc-seconds or approximately 1 Km^2 for the analysis.

Three scenarios for the distribution models were considered for this study: i) distribution of the sand fly species occurring in Bahia state, according to the monitoring survey; ii) distribution of *Lu. longipalpis*, the vector of VL in Bahia; iii) distribution of VL in the state considering disease incidence records from SINAN. The analysis was performed using maximum iterations of 500 with 75% of the data points for each species randomly selected as training points and used in model building and 25% of the records were test points used in model validation.

Table 5.1. Bioclim Variables - Variables are derived from Worldclim monthly climate data (http://www.worldclim.org, last accessed October 19, 2010).

Bioclim Variable	Code
Annual Mean Temperature	BIO1
Mean Diurnal Range (Mean of monthly (max temp - min temp))	BIO2
Isothermality (BIO2/BIO7) (*100)	BIO3
Temperature Seasonality (standard deviation *100)	BIO4
Max Temperature of Warmest Month	BIO5
Min Temperature of Coldest Month	BIO6
Temperature Annual Range (BIO5-BIO6)	BIO7
Mean Temperature of Wettest Quarter	BIO8
Mean Temperature of Driest Quarter	BIO9
Mean Temperature of Warmest Quarter	BIO10
Mean Temperature of Coldest	BIO11
Annual Precipitation	BIO12
Precipitation in Wettest Month	BIO13
Precipitation in Driest Month	BIO14
Precipitation Seasonality (Coefficient of Variation)	BIO15
Precipitation in Wettest Quarter	BIO16
Precipitation in Driest Quarter	BIO17
Precipitation in Warmest Quarter	BIO18
Precipitation in Coldest Quarter	BIO19

The model was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC), which computes the total AUC created by plotting sensitivity against the fractional predicted area for the species.

5.3. Results

Sand fly model - Thirty one sand fly species were identified in Bahia, but the ENM developed in this study was restricted only to species with ten or more presence records. Species that had no training data were by default removed from the analysis (Table 5.2).

A total of 15 species were modeled, of which 4 species (*Lutzomyia intermedia, Lu. longipalpis, Lu. migonei, Lu. whitmani*) are of medical importance. *Lu. fischeri* has not been proven as a vector, but this species is often found in households areas where cutaneous leishmaniasis is endemic (Brasil, 2007; Brasil, 2014).

Table 5.2. Number (N) and Relative Abundance (%) of Sand Fly Species Captured in Bahia - (Collection period July 2009 to December 2012).

Species	N[a]	%[b]
*Lutzomyia amazonensis**	1	0.001
*Lutzomyia ayrozai**	11	0.010
*Lutzomyia barrettoi barrettoi**	11	0.010
Lutzomyia capixaba	542	0.482
*Lutzomyia carrerai carrerai**	53	0.047
Lutzmoyia choti	121	0.108
*Lutzomyia cortelezzii**	2	0.002
*Lutzomyia davisi **	2	0.002
*Lutzomyia edwardsi**	1	0.001
Lutzomyia evandroi	1634	1.454
Lutzomyia fischeri	363	0.323
*Lutzomyia goiana**	107	0.095
Lutzomyia intermedia	16186	14.404
Lutzomyia lenti	488	0.434
Lutzomyia longipalpis	48083	42.788
*Lutzomyia longispina**	22	0.020
Lutzomyia migonei	1197	1.065
Lutzomyia oswaldoi	54	0.048
*Lutzomyia pascalei**	2	0.002
Lutzomyia pessoai	406	0.361
*Lutzomyia quinquefer**	5	0.004
*Lutzomyia salesi**	19	0.017
*Lutzomyia schreiberi**	2	0.002
*Lutzomyia serrana**	1	0.001
Lutzomyia shannoni	15	0.013
*Lutzomyia sordelli**	1	0.001
*Lutzomyia termitophila**	2	0.002
*Lutzomyia tupinambai**	4	0.004
Lutzomyia viannamartinsi	215	0.191
Lutzomyia whitmani	42622	37.928
*Lutzomyia yuilli yuilli**	2	0.002
*Lutzomyia sp.***	47	0.042
Not identified**	154	0.137
Total	112375	100

*Species that were removed from the models
**Not identified or identified to the genus only, not considered in the model
[a]Total number of specimens collected
[b]Relative abundance: nx/N * 100, nx: number of individuals belonging to species x, N: total number of sampled individuals.

Out of the 15 species modeled, three species did not result in significant prediction models (*Lu. choti*, *Lu. goiania*, *Lu. longispina*). The species models with their accuracy and significance are shown on Table 5.3.

Table 5.3. Area Under the Curve (AUC) of the Niche Models for the Sand Flies Species Captured in Bahia.

Species	Test AUC	*P* value
Lutzmoyia choti	0.9994	n.s
Lutzomyia capixaba	0.9778	<0.001
Lutzomyia evandroi	0.9931	<0.001
Lutzomyia fischeri	0.9973	<0.001
Lutzomyia goiana	0.9959	n.s
Lutzomyia intermedia	0.9755	<0.05
Lutzomyia lenti	0.9447	<0.001
Lutzomyia longipalpis	0.9565	<0.05
Lutzomyia longispina	0.9983	n.s
Lutzomyia migonei	0.9979	<0.05
Lutzomyia oswaldoi	0.9605	<0.001
Lutzomyia pessoai	0.9978	<0.001
Lutzomyia shannoni	0.9989	<0.001
Lutzomyia viannamartinsi	1	<0.001
Lutzomyia whitmani	0.982	<0.05

n.s.- not significant

Bioclim variables seemed to be more important factors in modeling sand flies in Bahia state than the vegetation indexes and land surface temperature data analyzed. In about 67% of the models, the most important variables were Bioclim variables while 34% of the models had NDWI, NDVI and LST day amongst the top four most important variables. A table of the variables that contributed to the models by species based on the Jackknife test is presented on Table 5.4.

The species presented various distribution patterns across the state. The predicted distributions for *Lu. capixaba* and *Lu. fischeri* followed the coast towards the south region of the state. *Lu. evandroi* and *Lu. longipalpis* were distributed throughout the state. *Lu. intermedia* and *Lu. migonei* were distributed in the central and southeast region of Bahia. *Lu. lenti*, *Lu. oswaldoi* and *Lu. pessoai* were mainly restricted to the central region of the state. *Lu. shannoni* was distributed throughout the southeast region. *Lu. viannamartinsi* was distributed towards the

south while and *Lu. whitmani* was distributed from the central region to the south of Bahia (Figure 5.2).

Mean diurnal range (BIO2) was the variable that most contributed in all the models for sand flies in Bahia, followed by precipitation in wettest month (BIO 13) (Table 5.4). The variables that added most information to the models, when analyzed individually, were mean diurnal range, precipitation in wettest month and precipitation in wettest quarter (BIO16). Temperature seasonality (BIO4) seemed to be the variable that decreased the AUC when omitted from most of the models.

Table 5.4. Environmental Contribution Based on the Jackknife Test - Highest contributing variable per model is depicted on the table. Highest gain represents the variable that adds more information to the model when used in isolation. Lowest gain represents the variable with most information that is not present in the other variables.

Species	% Contribution	Highest Gain	Lowest Gain
Lutzomyia capixaba	BIO2 62.8%	BIO2	BIO7
Lutzomyia evandroi	BIO13 59.7%	BIO13	BIO4
Lutzomyia fischeri	BIO2 68.5%	BIO2	BIO4
Lutzomyia intermedia	BIO2 32.5%	BIO16	BIO7
Lutzomyia lenti	BIO13 37.6%	BIO13	BIO8
Lutzomyia longipalpis	BIO12 29%	BIO13	BIO4
Lutzomyia migonei	BIO2 31.7%	BIO2	BIO4
Lutzomyia oswaldoi	BIO2 51.7%	BIO2	BIO4
Lutzomyia pessoai	BIO16 40%	BIO16	BIO4
Lutzomyia shannoni	BIO13 46.2%	BIO13	BIO4
Lutzomyia viannamartinsi	BIO2 66.8%	BIO2	BIO3
Lutzomyia whitmani	BIO2 61.2%	BIO2	BIO4

Lutzomyia longipalpis model – A total of 48,083 *Lu. longipalpis* specimens were captured in Bahia state between Jul, 2009 and December, 2012. *Lu. longipalpis* was the most prevalent species captured by LACEN. The relative abundance of this species was 42.78% (Refer to table 5.2).

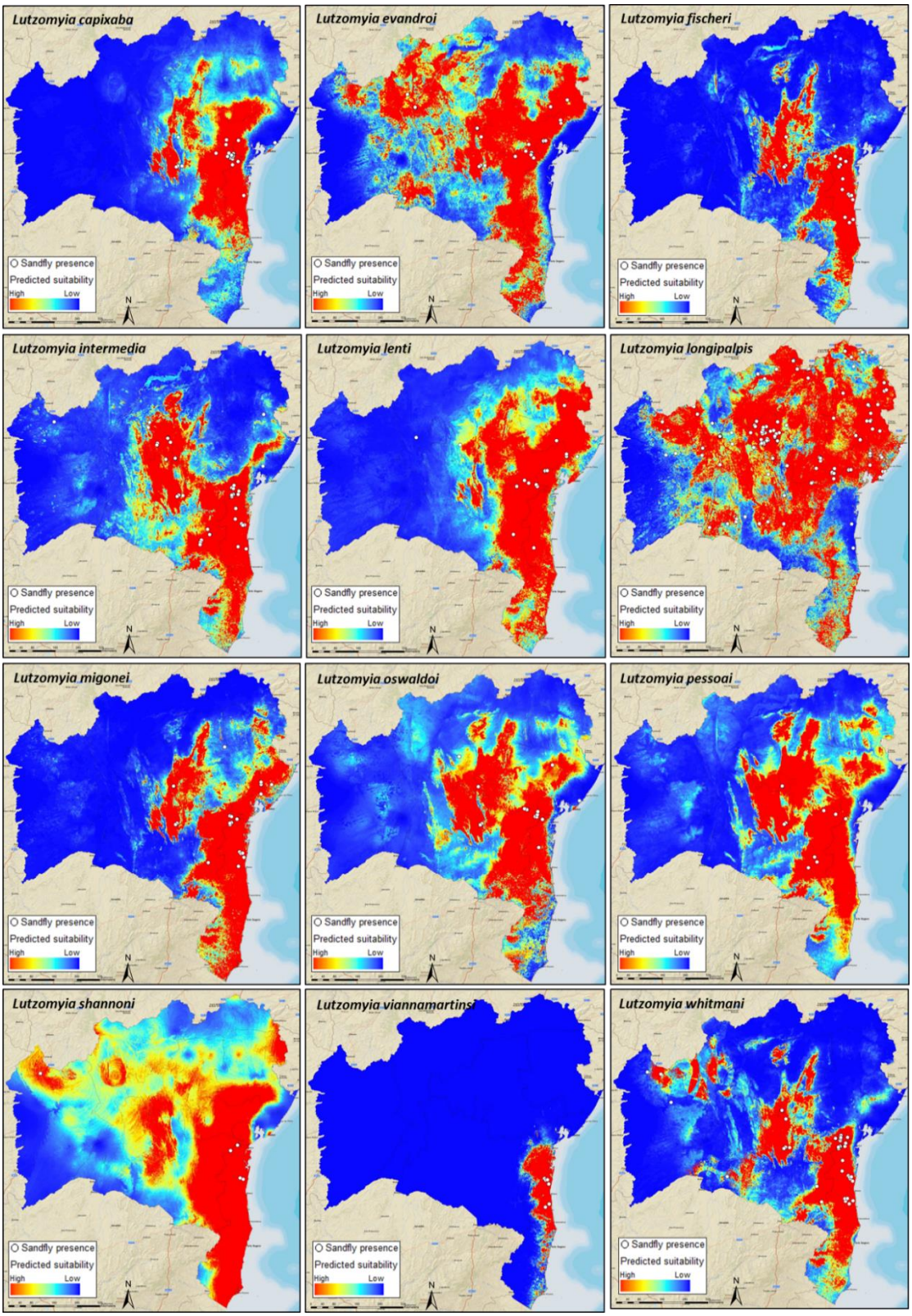


Figure 5.2. Maxent Predicted Distribution of Sand Flies Species Collected in Bahia State - Red areas indicate higher distribution probability.

Males were significantly more abundant than females (p=0.017), with an overall male/female ratio of 3:1. No significant differences were observed in sand fly density over the years (p=0.319) or monthly (p=0.360), despite a peak in density observed in December of 2011 to February, 2012 (Figure 5.3).

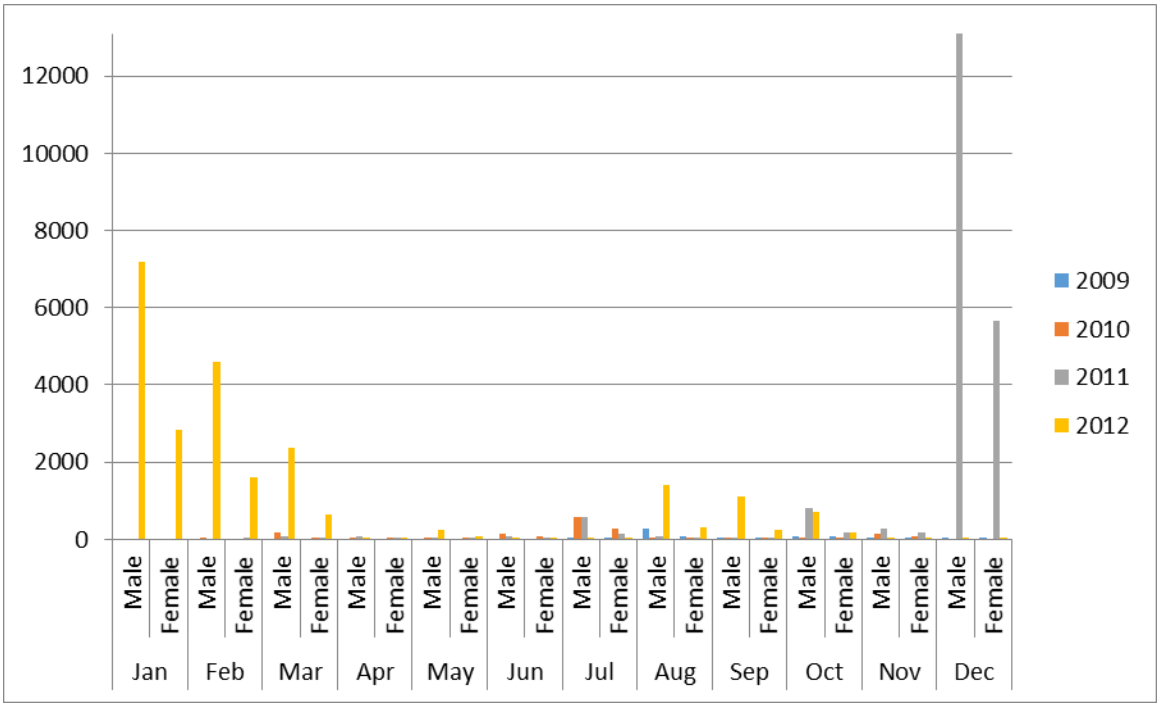


Figure 5.3. Monthly Distribution of Male and Female Sand Flies - Captured period from July 2009 to December 2012.

When a model for only the species *Lu. longipalpis* was developed, annual precipitation (BIO12), precipitation in wettest quarter (BIO16), precipitation in wettest month (BIO13) and NDVI were the variables that most contributed in the models (27.1%, 19.4%, 17.9% and 15.9% respectively) (AUC=0.972; p<0.001) (Figure 5.4). The environmental variable with highest gain, when used in isolation, was precipitation in the wettest month. The environmental variable that decreases the gain the most, when it is omitted, was temperature seasonality (BIO4). The predicted distribution probability for *Lu. longipalpis* in Bahia state is shown in Figure 5.5.

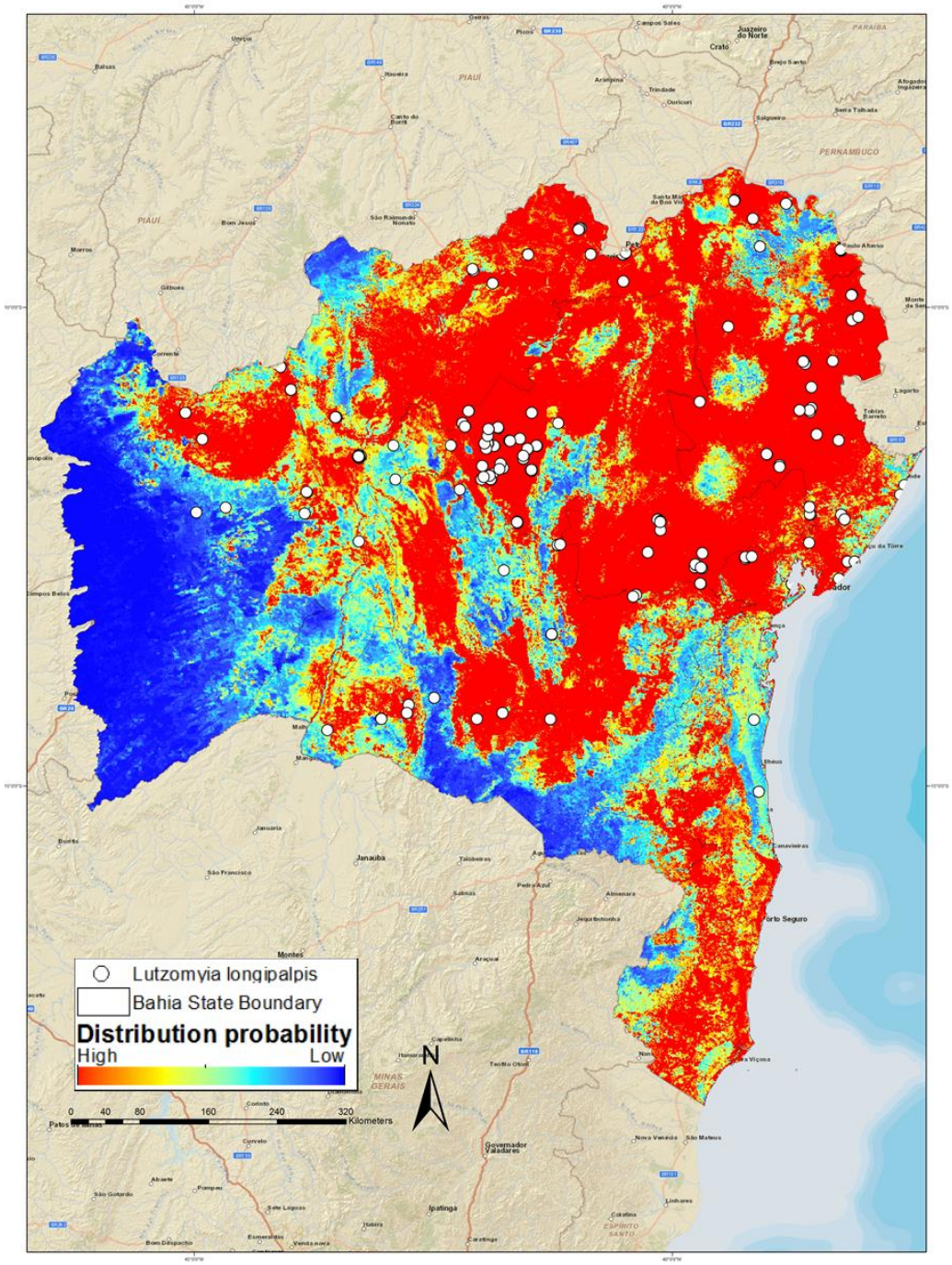


Figure 5.4. Maxent Predicted Distribution for *Lutzomyia longipalpis* in Bahia State - Red areas indicate higher distribution probability.

Visceral leishmaniasis model – Between 2009 and 2012, a total of 1493 cases of VL were recorded in Bahia. The disease was present in 56% (234/417) of the municipalities of the state. Approximately 13% (53/417) of these municipalities reported cases throughout the entire study period with an average of three percent of new areas being affected by VL. The year with the highest number of records of VL was 2010, but no significant difference between years was observed ($p>0.05$) (Figure 5.6). The spatial distribution of VL cases throughout the years is shown in Figure 5.7.

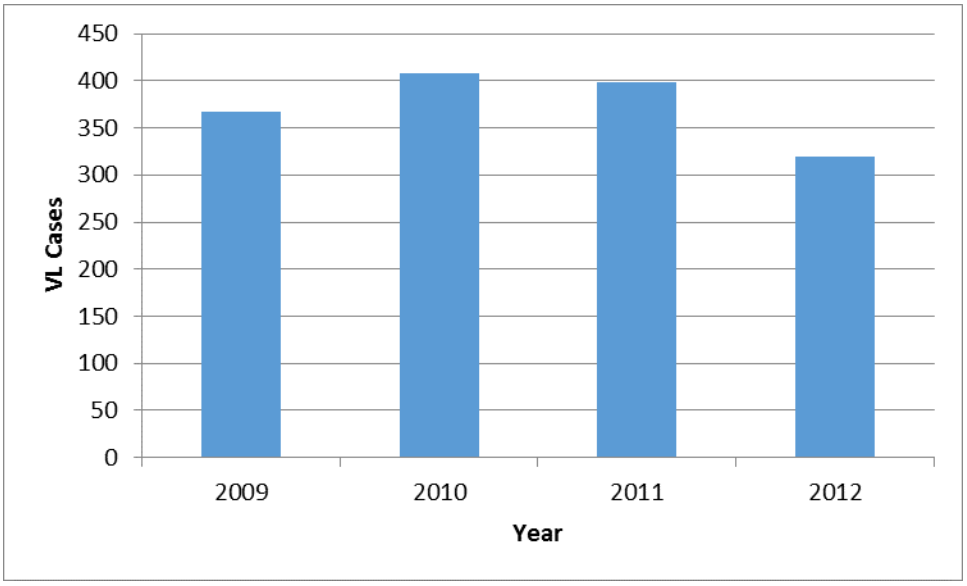


Figure 5.5. Yearly Distribution of VL Cases in Bahia State - No difference between years was noticed ($P>0.05$).

The Maxent model identified the central region of the state as more suitable for the occurrence of VL based on presence records for the study period (Figure 5.8). The variables that contributed the most in the model were precipitation in wettest month (BIO13), annual precipitation (BIO12), land surface diurnal temperature (LSTday) and temperature seasonality (BIO4) with 41.1%, 17.7%, 9.8% and 6% respectively.

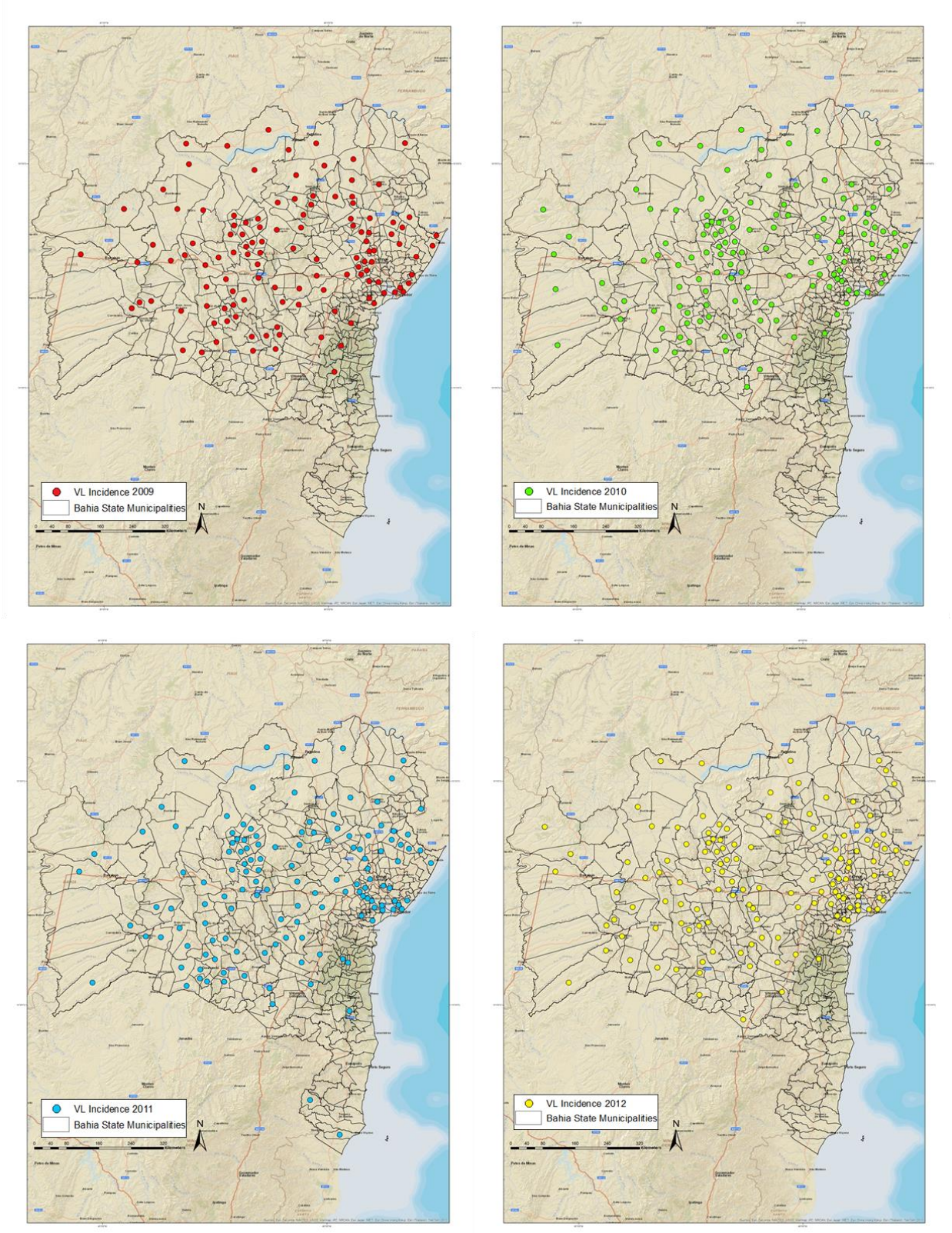


Figure 5.6. Spatial distribution of human cases of visceral leishmaniasis in Bahia state in 2009-2012.

Precipitation in wettest quarter (BIO16) was the variable that added most information to the model when isolated and land surface nocturnal temperature (LST night) was the variable that decreased the gain the most when omitted. The model had an AUC=0.984 and was statistically significant ($p<0.001$). A map of the potential distribution of VL in Bahia state is shown in Figure 5.7.

An area of overlap between the models of *Lu. longipalpis* and VL cases was detected. The northern, northeast and central regions of the state were suitable for the occurrence of *Lu. longipalpis*, but the areas suitable for VL cases do not extend to the northeast as it did for the vector. When superimposing the models of VL cases and *Lu. longipalpis*, considering a suitability greater than 0.60, the central and central east of Bahia state was shown to be suitable for the occurrence of vector and disease. Although the total overlapping area of disease and vector corresponds to only 1.11% of Bahia territory, the overlapping area included the municipalities that reported VL cases consecutively during the four years and the municipalities where the vector was captured throughout the study period (Figure 5.8).

5.4. Discussion

Phlebotomine sand flies are the only known vectors that can transmit the *Leishmania* parasites (WHO, 2011). VL has a transmission cycle that is based upon the dynamic interaction between the vector population and the population of mammalian hosts. Out of 31 sand fly species identified in Bahia, 12 species were modeled. Species with a small sample size could not be modeled because they did not have enough occurrence data, which could result in decreased predictive potential. Collections of phlebotomine sand flies tend to be associated with research related to human leishmaniasis and anthropogenic biomes and sometimes a large sample size is not possible to obtain. However it is important to consider that ENM accuracy increases with larger samples size (Colaccico-Mayhugh et al., 2010; Hernandez et al., 2006).

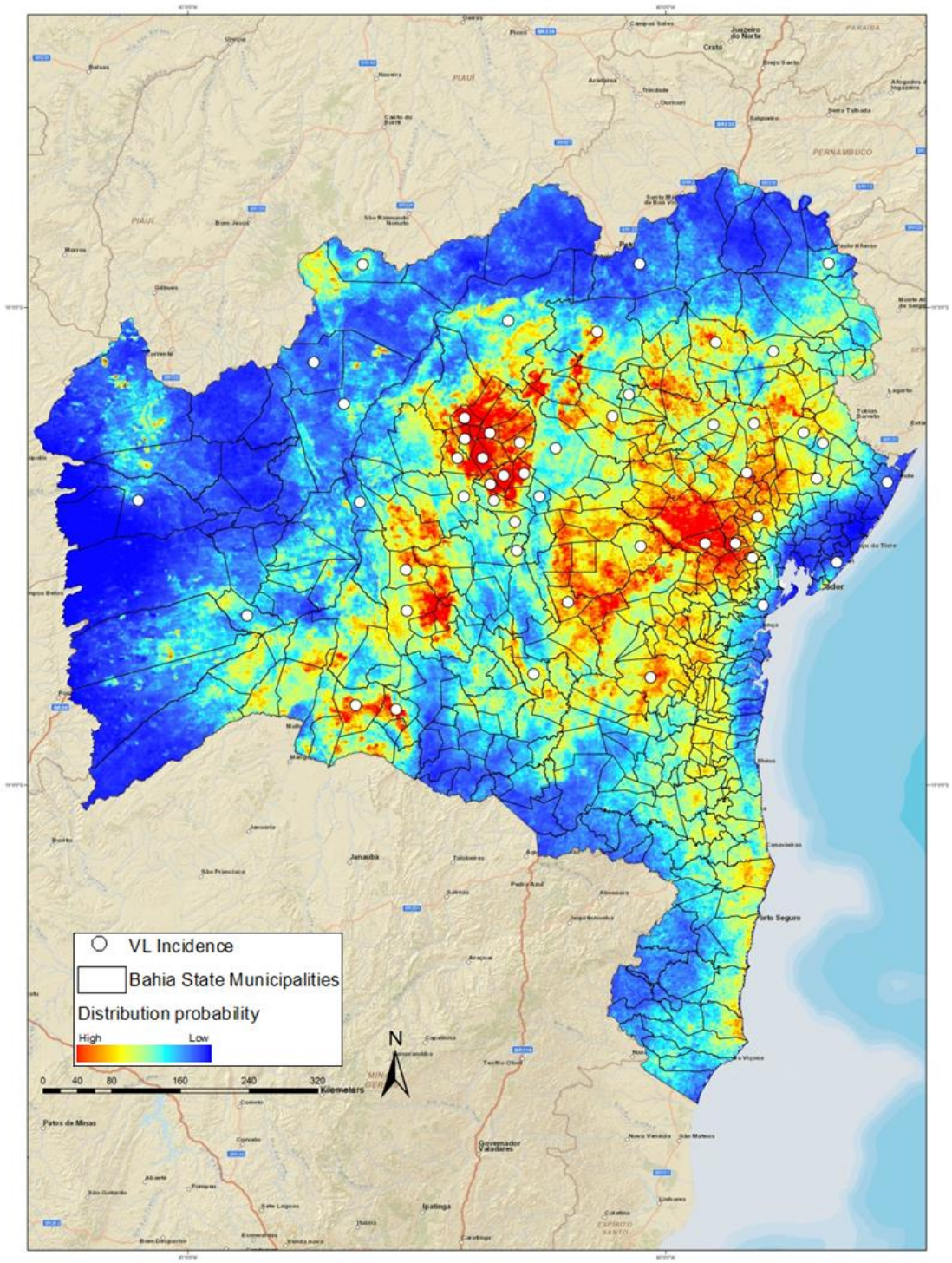
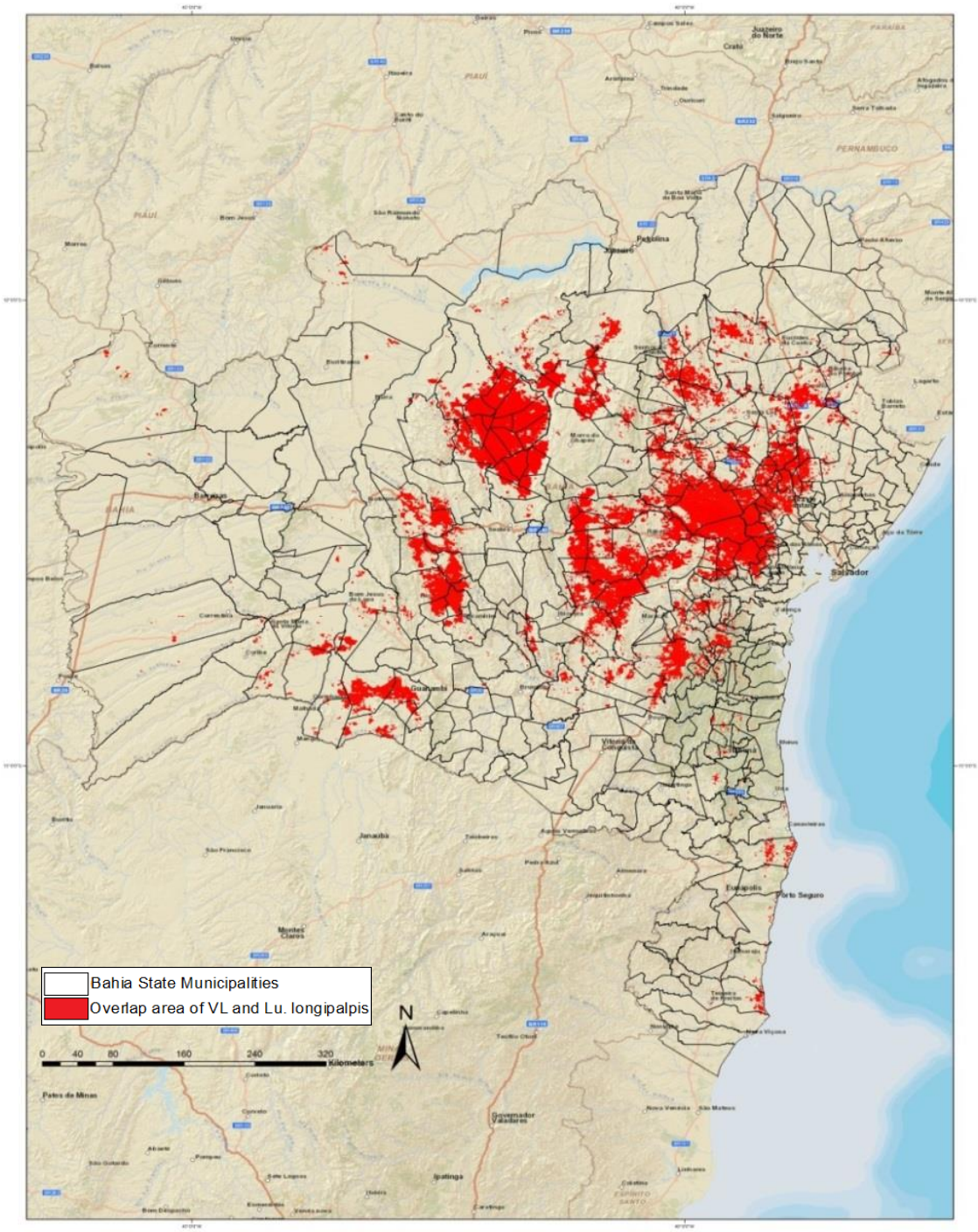


Figure 5.7. Ecological Niche Model for the Potential Distribution of Human Cases of VL - Areas identified as suitable based on locations where VL cases were consistently reported in all four years are presented on a blue to red scale: blue (low suitability) to red (high suitability).


Bahia State Municipalities
Overlap area of VL and Lu. longipalpis
0 40 80 160 240 320
Kilometers
N
Salvador
Porto Seguro
Ilhéus
Jequié
Barreiras
Juazeiro
Petrolina
Guanambi
Jacobina
Paramirim
Jaguaquara
Eunápolis
Teixeira de Freitas
Valença
Jequitinhonha
Jaguarari
Pedro Alexandre
Euclides da Cunha
Paulo Afonso
Santa Maria da Vitória
Santa Rita de Cássia
Formosa do Rio Preto
Correntina
Coribe
Cocos
Riachão das Neves
Mansidão
Buritirama
Canápolis
Ibipetum
Ibipetum
Ibipetum

Bioclim variables were more important factors in modeling sand flies in Bahia state than the vegetation indices and land surface temperature analyzed. Mean diurnal range was the variable that most contributed in the models. Mean diurnal range corresponds to the difference in day to night temperature and can be affected by relative humidity and cloud cover (Mischler et al., 2012). Highly variable temperature regimes can affect vector development. Temperature variability can be observed in arid and semi-arid areas of Bahia state, where most of VL cases have been reported (Franke et al., 2002). For mean diurnal range, habitat suitability for the species studied was low until the range begins to increase to approximately 9ºC and steadily increases to around 15 ºC showing that most sand fly species in Bahia have a stronger relationship to a moderate diurnal temperature range. Gonzalez et al. (2010), observed that the prediction distribution of leishmaniasis reservoirs species in North America, *Neotoma albigula*, was also influenced by mean diurnal temperature range.

Precipitation in wettest month and precipitation in wettest quarter were also important variables in the sand fly models. Gonzalez et al. (2014), found that the distribution of two VL vectors in Colombia, *Lu. longipalpis* and *Lu. evansi* was strongly correlated with precipitation. In Brazil it has been shown that precipitation, more than temperature, is the variable regulating local abundances of *Lu. longipalpis* populations (Queiroz and Varjão, 2012). In the present study, the limiting effect of precipitation during the wettest month and wettest quarter for the distribution of sand flies was when precipitation ranged between 10 and 80 mm. Rainfall above the tolerance limits of sand flies can interfere the reproductive cycles of these vectors (Queiroz and Varjão, 2012).

A 3:1 male/female ratio was observed. Similar findings have been reported in other studies (Almeida et al., 2010; Nunes et al., 2008; Resende et al., 2006). Male sand flies don't disperse very far. When male rates are higher than females it means that the area is closer to their habitat. *Lu. longipalpis* was the most abundant species found in the survey (42.78%). In the first years of the entomological survey beginning in 2009, small numbers of this species were found

with an increase in numbers by December 2011 to February 2012. This increase may be associated with intensification of surveillance or changes in environmental management (e.g., accumulation of organic matter, insertion of chicken coops and pig pens), that can favor vector establishment and dispersion.

Precipitation related variables (annual precipitation, precipitation in wettest quarter, precipitation in wettest month) and NDVI were the variables of most importance for *Lu. longipalpis* predicted distribution models. As previously mentioned, rainfall influences the dynamics, reproduction and breeding of vectors (Peterson & Shaw, 2003; Salomón et al., 2006; Quintana et al., 2010). A study of leishmaniasis vectors in Argentina showed that variables related to precipitation were the ones most influencing the risk models (Quintana et al., 2013). NDVI has been reported as an important risk factor for VL, associated with high numbers of sand flies and high incidence of human and canine cases of VL in the northwest region of Bahia (Bavia et al., 2005). Carneiro et al., (2004) observed a positive correlation between low NDVI, which represents areas of modified primary vegetation, associated with deforestation and the subsequent anthropic modifications and occurrence of VL in a central north area of Bahia. Colacicco-Mayhugh et al. (2010), identified high probabilities for the presence of *Phlebotomus alexandri* and *P. papatasi* in urban and field/woody savanna areas using land cover classes derived from NDVI and Bioclim within models generated using Maxent.

Precipitation related variables as well as temperature seasonality are indicative of areas with well-defined dry and rainy seasons. Temperature seasonality decreased the gain in all sand fly species models. Temperature seasonality is the standard deviation of the weekly mean temperatures and represents seasonal variations in temperature. Ecological studies of sand flies in Bahia have indicated that the population density of these insects, especially *Lu. longipalpis*, increases after the end of the wet season (Sherlock, 1996). Similar observations have also been made in the central region of Brazil (Almeida et al. 2010; Almeida et al., 2013; Oliveira et al. 2003). Almeida et al. (2013), observed that temperature seasonality and annual

precipitation were the most important variables in predicting the distribution of *Lu. longipalpis* in Mato Grosso, Brazil. Annual precipitation was the most important variable in prediction models of four sand fly species in Venezuela, including *Lu. longipalpis* (Sanchez et al., 2015).

For VL, variables related to precipitation and temperature (precipitation in wettest month, annual precipitation, land surface diurnal temperature and temperature seasonality) were the variables that most contributed to the model in the present study in Bahia. Sherlock (1996) observed that during dry periods, sand flies are more frequently found in the peridomicile or in the interior of houses, which increases the risk of VL incidence. Karangiannis-Voules found that the most suitable climatic and environmental factors for VL using a Bayesian approach in Brazil were low altitude, low annual precipitation, increased temperature diurnal range, and no extreme precipitation during the warmest quarter. Thompson et al. (2002), observed that the occurrence of VL in northeast of Brazil was cyclical and inversely related to a three-year-precipitation average.

A potential limitation of the niche model for both sand flies and VL cases in Bahia was the nature of data acquisition. The entomological data was not uniformly collected throughout the study period and the study area. Disease data location used the coordinates of the municipality administrative center. In order to perform more refined analysis, the point geographical coordinates of all confirmed VL cases may be necessary.

5.5. Conclusion

The present study shows the impact of environmental factors in vector and disease distribution in Bahia state, Brazil, identifying areas for occurrence of sand flies and VL in the state. Such models can represent a useful tool and contribute to the understanding of transmission dynamics of VL.

Bioclim variables were more important factors in modeling sandflies in Bahia state than the vegetation indices and land surface temperature analyzed. In particular, the mean diurnal range

corresponds to the difference in day to night temperature and can be affected by relative humidity and cloud cover. In semi-arid areas of Bahia, any variations in temperature regimes can affect vector development and lead to increase disease incidence. Precipitation is known to regulate local abundances of *Lu. longipalpis* populations. Rainfall above the tolerance limits of sand flies can interfere with vector development.

ENMs allows for a better understanding and identification of the geographic distribution of VL, either by the presence of its vector or the presence of diseases in humans or reservoirs and can also contribute to the planning of measures designed to control the vectors of leishmaniasis in Bahia.

Potential limitations of the study was the nature of data acquisition; the entomological data was not uniformly collected throughout the study period and the study area (collection based on human cases); disease data location used the coordinates of the municipality administrative center

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Chapter 6: Feeding Preferences, Natural *Leishmania* Infection and Ecological Niche of Phlebotomine Sand Flies in Monte Gordo, Bahia, Brazil

6.1. Introduction

Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania* Ross, 1903 (Kinetoplastida: Trypanosomatidae). These diseases are considered a serious public health problem in at least 88 countries, with approximately 350 million people at risk of contracting one of the clinical forms of leishmaniasis (Dujardin, 2006; WHO, 2011). Sand flies are the only known vector that can transmit the *Leishmania* parasite, particularly members of the genera *Phlebotomus* spp. and *Lutzomyia* spp. which are found in a wide range of habitats, from desert to tropical rain forest. In Brazil, the phlebotomine fauna is composed of more than 230 species. The principal species responsible for transmission of visceral leishmaniasis (VL) is *Lutzomyia longipalpis*, but there could be other species involved, as it has been seen in Mato Grosso State (Carvalho et al., 2007; Galati, 2003; Lainson and Rangel, 2005; Santos et al., 1998; WHO, 2011). This species is the most studied due to its epidemiological importance in the transmission of VL (Soares and Turco, 2003; Rangel and Vilela, 2008).

Vector adaptation to different habitats is probably the reason behind an increase in the incidence of disease in the past years and information regarding breeding habitat, feeding preferences and natural infection of sand flies is still scarce. *Lu. longipalpis* can be found in sylvatic and anthropic environments, in rural and urban areas and in any period of the year. This demonstrates great ecological flexibility of this species. This sand fly adapts easily to the peridomestic environment and is constantly found around the household, especially near to feeding sources (Neves et al., 2005) Overall, *Lu. longipalpis* is primordially a sylvatic species that can still be captured in remote primary forest that is far from human habitation (Lainson et al. 1986, Ryan et al. 1986; Teodoro et al., 1993). Native animals harbour *L. infantum* as an enzootic maintained by sylvatic sand flies (*Lu. longipalpis*) and the parasite inevitably gains

entrance into newly established human settlements. Infected sand flies that migrate from the nearby sylvatic enzootic, or infected scavengers such as foxes or opossums can come closer to human dwellings and be fed on by the sand flies from peridomestic populations (Lainson and Rangel, 2005). Despite being found in a variety of ecosystems, drastic environmental alterations have altered the ecology of some sand fly species as well as the epidemiology of leishmaniasis (Rangel and Vilela, 2008). When there is a retraction of the sand flies habitat by human occupation, it has been observed that the distribution of *Lu. longipalpis* matches the occurrence of VL cases (Soares et al., 2006).

Sand flies, as well as many other blood-sucking flies, need a supply of carbohydrates that, in nature, they acquire directly from plant sap, nectar (Alexander & Usma, 1994), aphid secretions and fruits (Cameron et al., 1995). Blood feeding is a unique behavior of females that need blood for the maturation of ovaries. Sand flies are versatile feeders and may take blood meals from a wide variety of hosts, including reptiles, amphibians, human, livestock, dogs and chickens (Lainson and Rangel 2005; Magnarelli & Modi, 1988; Van Handel, 1984). Host-feeding patterns and preferences vary according to a number of innate, seasonal, and environmental trends, which may include host availability and abundance, flight behavior, feeding periodicity as well as geographic region (Molaei et al. 2008; Turell et al. 2005).

The study of gut contents of hematophagous insects is an alternative to knowing which domestic and sylvatic animals are effectively used as blood meal host in an anthropic environment. The anthropophilic index is a vital component of vectorial capacity. Detailed knowledge of the feeding behavior of sand flies on their various vertebrate hosts and identification of an animal reservoir as well as assessment of vector infection in a vector-borne disease transmission cycle is critical for the establishment of an efficient control strategy (Boakye et al., 1999).

Molecular techniques have been utilized as a tool for a quick, sensitive, and specific diagnostic in the study of leishmaniasis. Polymerase chain reaction (PCR) identification of

vertebrate host blood meals is a potentially convenient alternative, which has already been performed on several vectors including ticks (Pichon et al. 2003, Estrada-Peña et al. 2005), triatomine bugs (Bosseno et al. 2006, Pizarro et al. 2007) and mosquitoes (Watts et al., 2009). PCR has also been described in a variety of studies as effective in detection and identification of *Leishmania* (Paiva, 2009; Uezato et al., 1998). The results obtained by PCR for detection of *Leishmania* are not dependent on the quantity of parasites present in the samples (Quaresma et al., 2012; Dougall et al., 2011; Marcelino et al., 2011; Pita-Pereira et al., 2011; Vasconcelos et al., 2011).

PCR based on primers designed from multiple alignments of the mitochondrial cytochrome *b* gene have identified avian and mammalian hosts of various species of mosquito to the species level, which can be achieved with much higher accuracy than previously used serology techniques (Alcaide et al., 2009; Kent et al. 2006; Molaei et al., 2008; Townzen et al., 2008; Valinsky et al., 2014). Lassen et al. (2011), used the cytochrome *b* gene amplification to identify the vertebrate hosts of blood-fed female biting midges (Diptera: Ceratopogonidae: *Cullicoides* Latreille) and were successful in 90% of the samples analyzed. Techniques used to identify host preferences include group specific PCR, PCR followed by DNA sequencing, PCR–restriction fragment length polymorphism (PCR-RFLP), PCR followed by reverse line blot hybridization (RLB), real-time PCR, heteroduplex mobility assays, and multiplex PCR (Abbasi et al., 2009; Kent, 2009; Paiva-Cavalcanti et al., 2010; Sant'Anna et al., 2008).

For natural infection by *Leishmania spp*. one target sequence usually chosen for PCR analysis is the kinetoplast minicircle DNA (kDNA). The kDNA is present in *Leishmania spp*. in large quantities and has a preserved region of at least 120 base pairs that can be found in each molecule (Maia et al., 2013; Rodgers et al., 1990; Weigle et al., 2002). Other sequence products used for identification of Leishmania parasites include ribosomal RNA genes, heat shock protein 70 kDa (*hsp70*), glucose-6-phosphatase desidrogenase gene and ribosomal internal transcribed

spacer 1 region (Castilho et al., 2003; Quaresma et al., 2012; Uliana et al., 1994; Valinsky et al., 2014).

As important as identifying the feeding preferences of vector of diseases, is identifying the environment in which vectors thrive and can be found. Ecological niche models (ENM) estimate the relationship between species records at sites and the environmental and/or spatial characteristics of those sites (Franklin et al., 2009). A variety of methods have been used to predict species potential distribution using ENM based on occurrence data points and environmental variables, with generally good results (Peterson, 2006; Tsoar et al., 2007). ENM can be significant in vector studies, suggesting potential areas of vector occurrence, and consequently identifying risk areas of pathogen transmission (Peterson, 2006). Most of the ENM for diseases occurrence however, have been developed at country or state scales (Ceccarelli et al., 2015; Karagiannis-Voules et al., 2013; Quintana et al., 2013; Sanchez et al., 2015). In most cases, depending on the territorial extension of the study area (i.e., country, state or even municipality), it can be divided into different ecological zones and some models might not be able to properly capture the geographical distribution of vectors or diseases. Mapping potential habitats using a finer spatial scale could substantially improve the temporal and spatial resolution risk maps and create novel possibilities for improved disease management based on better understanding of transmission dynamics at the local habitat scale (Charlier et al., 2011).

Worldview-2 (WV-2) is a high resolution 8-band (four standard colours, wavelengths: blue, 0.45-0.52 µm; green, 0.52-0.59 µm; red, 0.62-0.68 µm; and nearinfrared 1, 0.77-0.86 µm, and four new colours, wavelengths: coastal, 0.40-0.45 µm, yellow, 0.58-6.25 µm, red edge, 7.05-7.45 µm, and near-infrared 2, 8.60- 10.45 µm), satellite. It provides a multispectral resolution of 1.85 meters and a temporal resolution of 1.1 days (www.digitalglobe.com, 2010). WV-2 has been widely used in agricultural and epidemiological applications. Ehlkes et al. (2014), used WV-2 imagery to study the effects of land cover and land use in malaria (*Plasmodium falciparum*) transmission in Ghana and showed the importance of assessing

small-scale spatial variation of features such as vegetation to circumvent generalized assumptions on ecological associations. De Roeck et al. (2014), successfully detected and characterize small water bodies associated with *Galba truncatula*, the snail vector of *Fasciola hepatica*, habitats in Belgium using disease case points and WV-2. Risk and protective environmental factors have been identified for the presence of *Aedes aegypti* in human dwelling using high resolution imagery (Geoeye-1) in Martinique (Machault et al., 2014). By using high relosuiton images they were able to produce risk maps at the household level with daily temporal resolution. Such findings can contribute to targeted operational control programs. The results of studies using high resolution images indicate that vector habitats can be detected at a high spatial resolution investigation and such techniques represent an asset in vector-borne diseases research and improve risk mapping for infections and risk management.

Bahia is one of the states in Brazil with the highest record of visceral leishmaniasis (VL) (Brasil, 2014). In the metropolitan areas of the state, Camaçari is the municipality with the highest number of reported cases in humans and dogs. This municipality is composed of three districts: Abrantes, Camaçari and Monte Gordo. The district of Monte Gordo is primarily rural and originated from a farm named after its owners (Padilha et al., 2006). This area has been experiencing accelerated growth in the past several years. Besides the main agricultural activity, Monte Gordo attracts individuals from other areas for tourism (i.e. beaches, fishing). Monte Gordo is in a privileged location, with easy access to the coast and the largest chemical plant in the state. It is also considered a trendy area with food outlets, various services and growing commerce. This region has been classified as moderate to intense for the transmission of VL according to the guidelines of the Brazilian Ministry of Health (Brasil, 2007; Brasil, 2014). The Entomology Sector of the Central Laboratory (LACEN) has selected the municipality of Camaçari to be part of the monitoring activities of the entomological surveillance program. Visceral leishmaniasis is endemic in the entire municipality of Camaçari and due to its economic

importance, the district of Monte Gordo has been selected for the study on blood meal identification and natural *Leishmania* infection of sand flies.

The objectives of this study was to detect feeding preferences and natural *Leishmania* infections in sand flies collected in Monte Gordo, Bahia State, Brazil while producing a practical methodology that can be incorporated into the monitoring activities performed by the Entomology Sector of LACEN; and identify priority areas for intervention based on a community level ENM of sand flies based on high resolution WorldView2 imagery.

6.2. Material and Methods

Monte Gordo is a district that belongs to the municipality of Camaçari, located in the metropolitan region of Salvador (Figure 6.1).

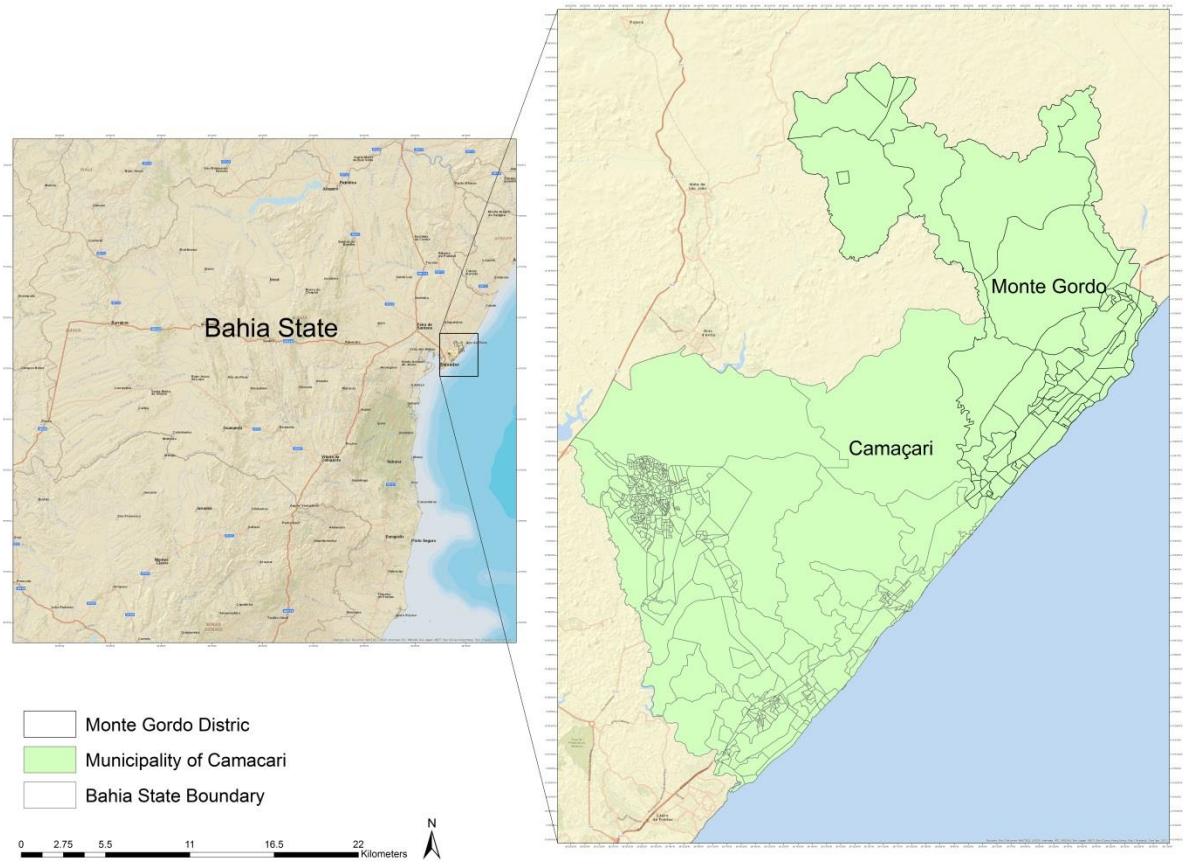

Figure 6.1. Study Area, District of Monte Gordo, Camaçari, Bahia.

Monte Gordo is located on 12º 39’ 00” S and 38º 4’ 00” W. The main access to Monte Gordo is by the BA-099 and the city is 66.4 kilometers from Salvador, the capital of the state (Sena, 2010). The area is primarily rural with a population of 29,573 inhabitants, 7.93% of the total population of the municipality, in a territorial area of 287 Km^2. The climate is tropical with significant precipitation throughout the year, even during the dry season. The mean temperature for the area is 24.7°C and has an annual precipitation of 1713 mm (IBGE, 2010).

Sand fly collections were performed in conjunction with the monitoring surveys carried out by the Entomology Sector of LACEN. The monitoring survey was carried out for a period of two years, and the captures for the purpose of blood meal and *Leishmania* infection identification were carried out over a period of three months, from June to August, 2013. CDC light traps were placed in the peridomicile area of known locations of VL cases in humans and dogs. The traps were placed every month for four consecutive days from 6pm to 6am. The insects captured were killed in ethyl acetate and then screened using a stereoscopic microscope to separate sand flies from other insects. Sand flies were preserved in 70% ethanol and maintained at room temperature for further analysis. Sand flies were then separated by gender and female sand flies were classified as engorged if they had blood in the abdomen or unfed if there was no visible blood in the abdomen. Species identification was done by morphological characteristics of the head and genitalia using the taxonomic key of Galati (2003).

Species were prepared for identification following the guidelines of LACEN. Males were subjected to clarification in three steps: i) incubation in 20% potassium hydroxide solution for 24 hours; ii) incubation in 10% acetic acid solution for 40 minutes with three washings in distilled water, and 15 minutes immersion in each wash, iii) incubation in lacto-phenol for 24 hours. After clearing, the male sand flies were placed on a microscope slide and examined under a stereoscopic microscope for morphologic evaluation. For females, the heads and genitalia were

dissected and mounted on a microscope slide with Berlese's fluid, covered with a coverslip and examined under a stereoscopic microscope for morphologic evaluation.

The abdominal segment of blood fed females was physically triturated in 20 µL of a buffer solution of phosphate-buffered saline (PBS, pH 7.2 --- 50 mM potassium phosphate, 150 mM NaCl) and stored in a 2 ml microcentrifuge tubes at -20°C for 24 hours. The samples were allowed to thaw at room temperature and 180 µL of PBS was added to the microcentrifuge tubes and DNA extraction was performed.

DNA extraction was done using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA) and carried out according to the manufacturer's protocol with a slight modification in the elution step. The elution step was performed twice, each time a volume of 70 µl of Buffer AE (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0) was used. The purpose of this modification was to allow for higher DNA yield. Extracted DNA from each sample was quantified by using Nanodrop.

Food source identification was performed via PCR using the modified vertebrate-universal specific primers previously described (Steuber et al. 2005). A 359-basepairs fragment of the cytochrome b gene (*cytB*) was amplified using the *cytB* forward primer, 5'-CCATCCAACATCTCAGCATGATGAAA-3' and the *cytB* reverse primer, 5'-GCCCCTCAGAATGATATTTGTCCTCA-3'. For natural *Leishmania* infection, amplification of a 447-bp fragment of kinetoplastid DNA minicircle was carried out using the MC1 forward primer, 5'-GTTAGCCGATGGTGGTCTTG-3' and the MC2 reverse primer 5'-CACCCATTTTTCCGATTTTG-3'.

Amplification reactions were prepared in 12.5µL of 1x buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 0.25µL of forward primer (20 mM), 0.25µL of reverse primer (20 mM), 0.25µL of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA) and 11.75µL of DNA template (average of 60ng/µL sample DNA) in a final volume of 25 µL. Positive controls included sand flies from a laboratory colony artificially fed on blood from human, avian, canine and swine hosts for the blood meal analysis and canine blood infected with leishmania for natural infection.

PCR was performed in an automated thermal cycler with the following cycling conditions: 30 cycles of 95ºC for 15 minutes, 94ºC for 1 minute, 55ºC for 1 minute and 72ºC for 1 min, followed by a final extension step at 72ºC for 10 minutes.

Amplified PCR products were analyzed on a 1% agarose gel stained with 4µL of DNA running dye for separation of amplicons. Excised bands were stored at 4ºC until DNA purification. Fragments were sequenced on both strands with BigDye Terminator Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, CA) at the Gene Lab at the School of Veterinary Medicine, Louisiana State University. Sequences were analyzed and edited with Sequencher TM software (Gene Codes Co., Ann Arbor, MI), and were compared with the GenBank database entries by the BLAST program from NCBI (http://blast.ncbi.nlm.nih.gov). A brief schematization of steps in the analysis of stomach contents of sand flies is shown in Figure 6.2.

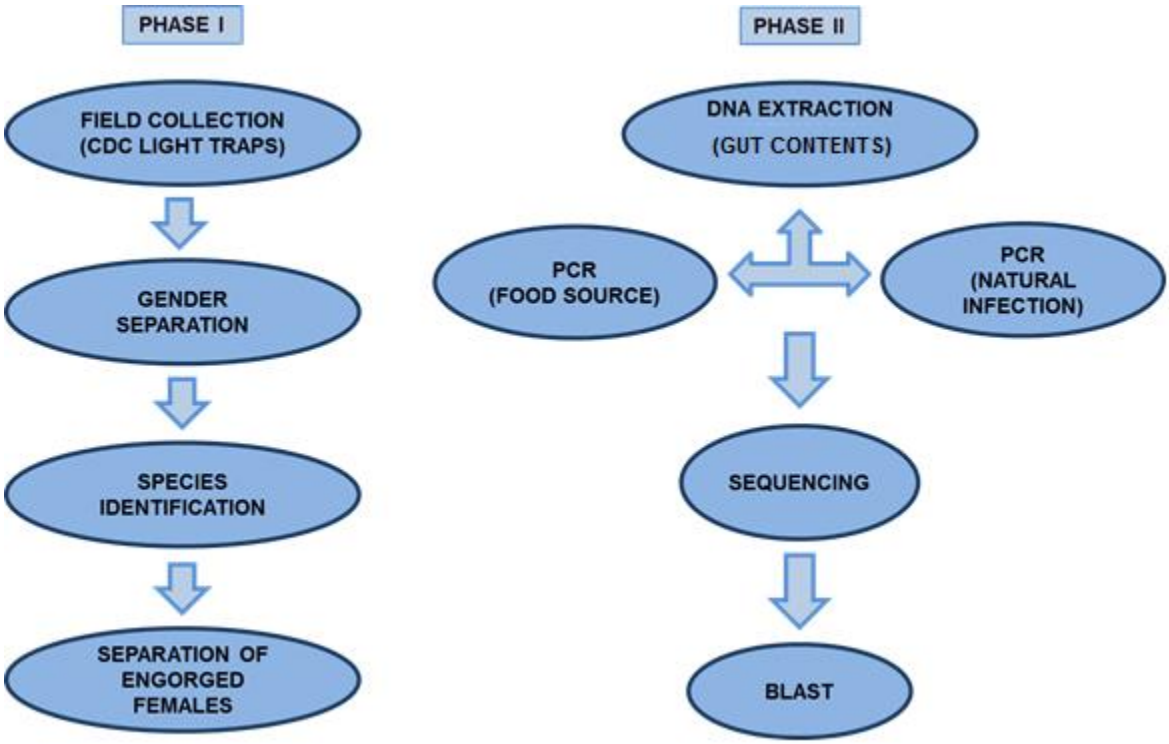

Figure 6.2. Schematization of Processing Sand Flies Captured in Monte Gordo, Bahia for Identification of Food Source and Natural Infection by *Leishmania* spp.

Ecological Niche Model: A WV-2 image from April 2013 was used to derive three vegetation indexes to be considered in the ENM for sand flies in Monte Gordo. The vegetation indexes used were NDVI, NDWI and Normalized Difference Soil Index (NDSI), previously described in Chapter 4. Index ratios are usually used to classify a specific feature by identification of abundance, health, condition and variety of surface materials. A special consideration about the indexes derived from WV-2 images is that the NDWI uses the coastal band and identifies areas of standing water. The NDVI uses a NIR2 band which has a higher reflectance than traditional NIR bands, thus producing a higher NDVI value. The NDSI identifies areas where soil is the most dominant background material. Instead of using a SWIR band, which is not present in WV-2 images, the green and yellow band are used as a way of exploring soil due to the unique response values that those bands produce regarding soil features. The band ratio calculation is illustrated in figure 6.3 and the bands used are as follows:

$$NDVI = {NIR2\ (Band\ 8) - Red\ (Band\ 5)}\Big/{NIR2(Band\ 8) + Red\ (Band\ 5)}$$

$$NDWI = {Coastal\ (Band\ 1) - NIR2\ (Band8)}\Big/{Coastal\ (Band1) + NIR2\ (Band\ 8)}$$

$$NDSI = {Green\ (Band\ 3) - Yellow\ (band4)}\Big/{Green\ (Band\ 3) + Yellow\ (Band4)}$$

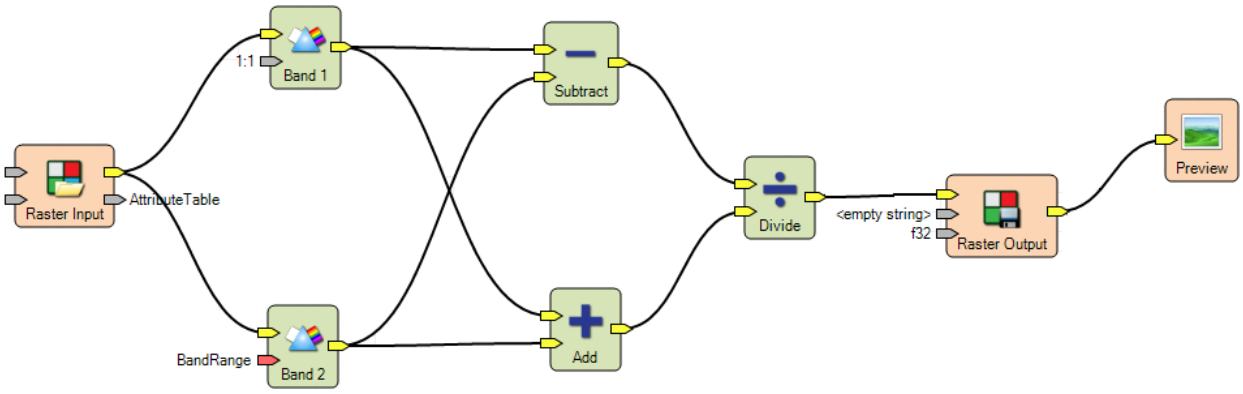

Figure 6.3. Model Building Steps for Deriving the Vegetation Indexes from WV-2.

Bioclim variables were also used for construction of the ENM for Monte Gordo. Both Bioclim variables and the vegetation indices were resampled to the same grid resolution as WV-2 (approximately 2 meters) for the analysis. The ENM for sand fly potential distribution in Monte Gordo was then built in Maxent using a maximum iteration of 500 and 25% of the data set aside for testing the accuracy of the model. The model was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC),

Data from the vegetation indexes was also extracted using buffers of 250 and 500 meters around the location of the CDC traps. The buffer size was selected based on the minimum dispersion range for the sand fly. Pearson's correlation and linear regression were used to analyze the variables associated with sand fly density in Monte Gordo (Figure 6.4).

6.3. Results

The collection sites were characterized as a rural area with transitional vegetation surrounding human dwellings (Figure 6.5). Sand flies were found in all locations where the CDC light traps were placed. The only species identified was *Lutzomyia longipalpis*. Besides humans, chickens, dogs and cats were the most common animals observed in the collection sites, with chickens and dogs being present in all the collection sites. In one of the locations there was a pig pen and some horses. A CDC trap was placed in the pig pen and it was the site with the highest sand fly density during the monitoring period of two years by LACEN.

During the collection period for the present study, the pig pen was empty, but sand fly capture was still possible in that location. The number of sand flies collected and available hosts per collection site is shown in Table 6.1. Figure 6.6 illustrates the animals observed during placement and retrieval of CDC light traps.

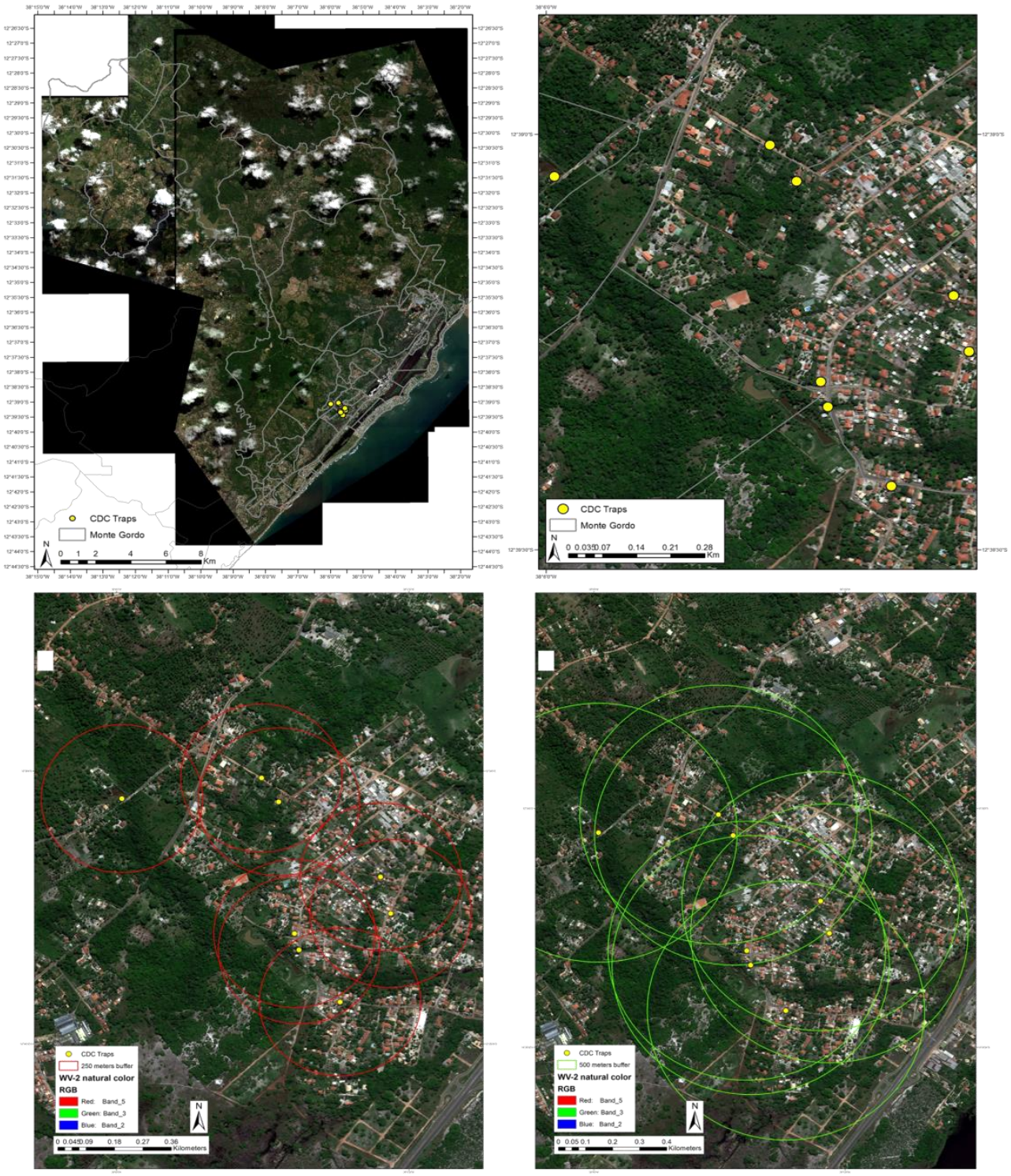

Figure 6.4. WordView-2 Imagery Composite for Monte Gordo - Top right: imagery composite covering the district of Monte Gordo, Bahia. Top left: Closer up of the CD trap locations. Bottom right: Buffer zone of 250 meters around the CDC trap. Bottom left: Buffer zone of 500 meters around the CDC traps.

Figure 6.5. Characteristics of the Collection Sites - From left to right: CDC light trap used for the collections, households where the traps were placed in the peridomicile and animal shelter areas.

Table 6.1. Characteristics of the Sand Fly Collecting Sites Regarding Host Availability.

Trap	Altitude (m)	Available hosts (within 50m)	Number of Sand flies
1	27	Humans, chickens, dogs, cats	49
2	27	Humans, chickens, dogs, horses	11
3	19	Humans, dogs, pigs, horses	21
4	24	Humans, chickens, dogs, cats	51
5	20	Humans, chickens, dogs, cats	19
6	18	Humans, chickens, dogs, cats	9
7	18	Humans, chickens, dogs, cats	4
8	15	Humans, dogs,	1
9	11	Humans, chickens, dogs	7
10	9	Humans, chickens, dogs	20

Figure 6.6. Predominant Available Hosts within 50 meters of the Collection Sites - Besides humans, chickens and dogs were present in all the locations where sand flies were captured.

A total of 192 sand flies were captured during the three month period. The flies were grouped into 40 pools based on location and day of capture. The only species captured was

Lutzomyia longipalpis. Males were significantly more abundant than females ($p<0.001$), with an overall male/female ratio of 2.84:1.

Fifty percent of the pools had engorged females, however only 40% of these pools had enough material to yield DNA amplification. Identification of blood meals and natural infection could not be accomplished in 16 of the pools because of failure in PCR amplification due to small quantities of DNA in the sample or and/or low sequence quality, even after several amplification attempts.

Natural infection by leishmania parasites was not observed in any of the engorged females tested. Successful identification of blood meal source was obtained in about 38% of the engorged females tested. Species identification via DNA sequencing determined that there was always one host per blood meal. Only two hosts were identified in the blood meals extracted from *Lu. longipalpis* collected in Monte Gordo. Domestic chicken (*Gallus gallus domesticus*) was the predominant host in the blood meals (67%) followed by human blood (Figure 6.7).

The ENM for sand fly potential distribution in Monte Gordo showed that temperature annual range (63.3%) was the variable that most contributed to the model, followed by precipitation in driest month (13.6%), mean temperature of coldest quarter (10.7%) and Normalized Difference Soil Index (NDSI) (4.4%). Sand fly suitability tended to decline if the temperature annual range rose above 10.2ºC. A similar trend was observed in relation to the precipitation in driest month. There was a gradual decline in sand fly distribution probability at rainfall levels above 70 to 73.5 mm when the distribution probability then becomes 0. When the mean temperature of coldest quarter is greater than 22.4ºC, sand fly suitability increases and the distribution probability became constant at 23ºC.

For NDSI, sand fly suitability increased as the soil content in the area increased. The variable with highest gain, when used in isolation, was temperature annual range, and the mean temperature of the driest quarter was the variable that contained more information that is not

present in the other variables analyzed; thus when this variable was removed from the model, the gain decreased (AUC=0.958; p<0.001) (Figure 6.8).

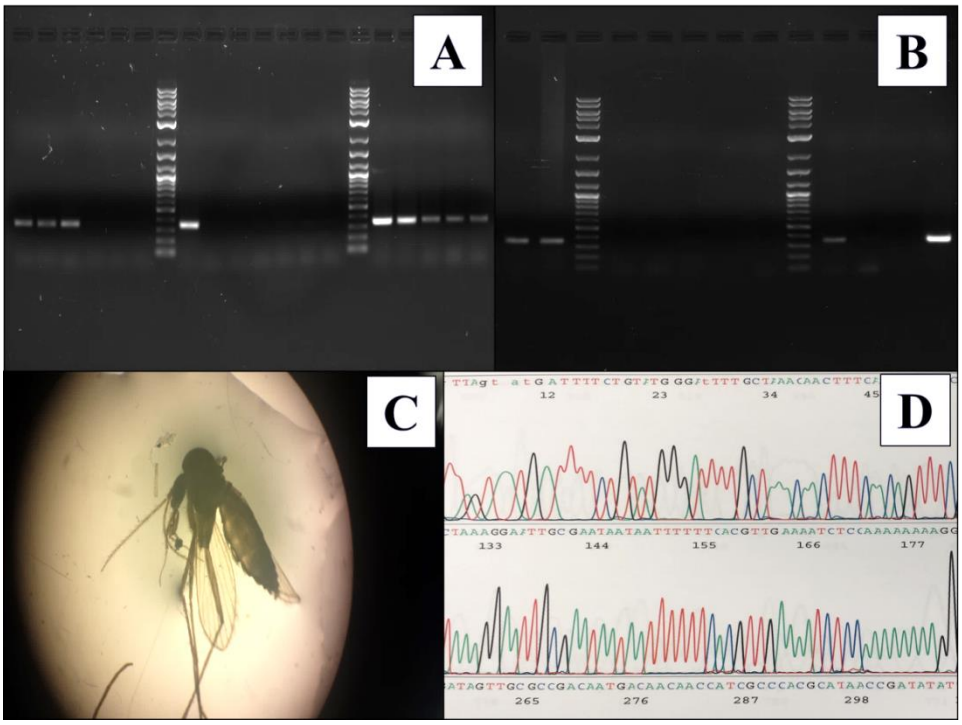

Figura 6.7. PCR Amplification and Sequencing Product from DNA Extracted from Engorged Sand Fly - PCR gels with the amplified bands for food source identification (A, B); engorged female Sand Fly (C); excised bands sequencing (D).

The mean temperature recorded during the three month collection period was 24ºC. No correlation between temperature and sand fly density was observed (p>0.05). The altitude where the CDC traps were placed ranged from 9 to 27 meters above sea level. A positive correlation between sand fly density and altitude was observed (p=0.054). The mean NDVI observed for the area where the traps were placed was 0.539, which represents a transition area between man-made features and some vegetation biomass. The mean NDWI observed was -0.279. NDWI values close to 1 indicate water bodies. Value closer to -1 may indicate man-made features or bare soil. The mean NDSI was -0.139. Positive NDSI values indicate soil features while negative values are an indicator of water bodies or vegetative features. In the study area it was also observed that some rooftops have the same values of soils if they have

clay tile roofs. The mean soil values observed indicated a transitional area of vegetation and man-made materials. No significant difference was observed between the vegetation indexes and location of traps (Figure 6.9). An inverse relationship between sand fly density and NDVI within a distance radius of 250 m was observed (p=0.028). For all other indices and buffer distance categories, no relationship with insect density was observed (p>0.05).

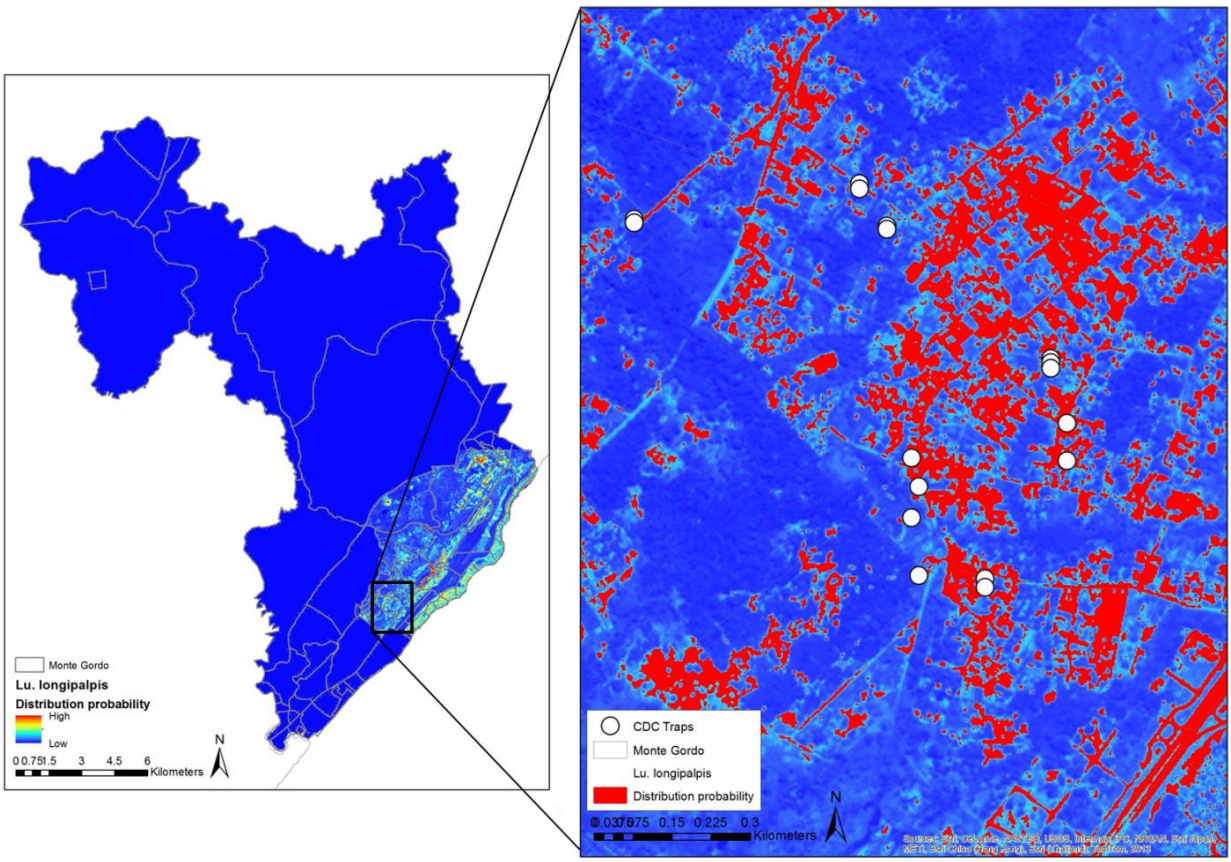

Figure 6.8. Predicted Suitability of *Lutzomyia longipalpis* in Monte Gordo Based on CDC Trap Locations and Environmental Variables - Red color indicates higher suitability, blue color depicts unsuitable areas.

A model considering only the vegetation, soil and water indices was developed for this scale and the surface suitable area for *Lu. longipalpis* in Monte Gordo is shown in Figure 6.10. Vegetation (NDVI) is the most important variable, contributing with 99.4% in the model while NDSI contributed with 0.6% and NDWI did not contribute in the model at al (0%) (AUC= 0.818; p<0.001). The imoirtance of vegetation has been described in the literature. In the areas with

the highest probability distribution for *Lu. longipalpis* it is possible to observe the transition environment of recently occupied areas.

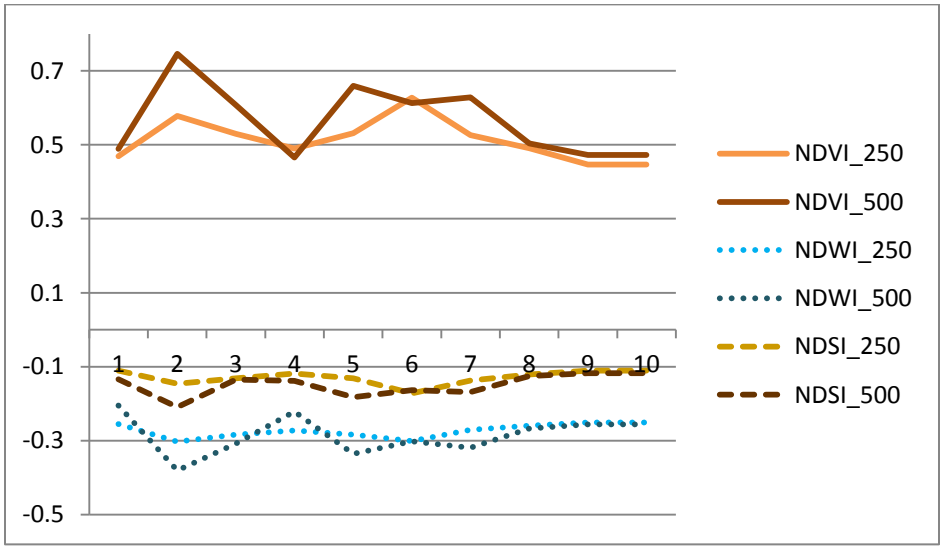

Figure 6.9. Mean Vegetation Indices Extracted from Buffer Distances of 250 and 500 meters Around the Collection Site - No significant difference was observed between the vegetation indices and location of traps.

As the soil features increase in the area, the probability for Lu longipalpis occurrence decreases, could be explained by the fact that the vector still needs the vegetation and moisture environment required for its maintenance. As it has been mentioned, NDWI did not contribute in the models. The water index derived in WV-2 is used to identify water bodies. For *Lu. longipalpis* this index is not important considering that sand flies have terrestrial larvae, so water is only important in the sense of prociding the overall environmental moisture necessary for development. But this index had shown promising results in identifying at higher resolutions habitats for other vectors such as the snail vector of *Fasciola hepatica* in Belgium for instance (De Roeck et al., 2014).

Most of the Ecological Niche Models (ENMs) have been developed at either the national, state scale or even regional level, but this is the first time the ENMs have been done at the community level.

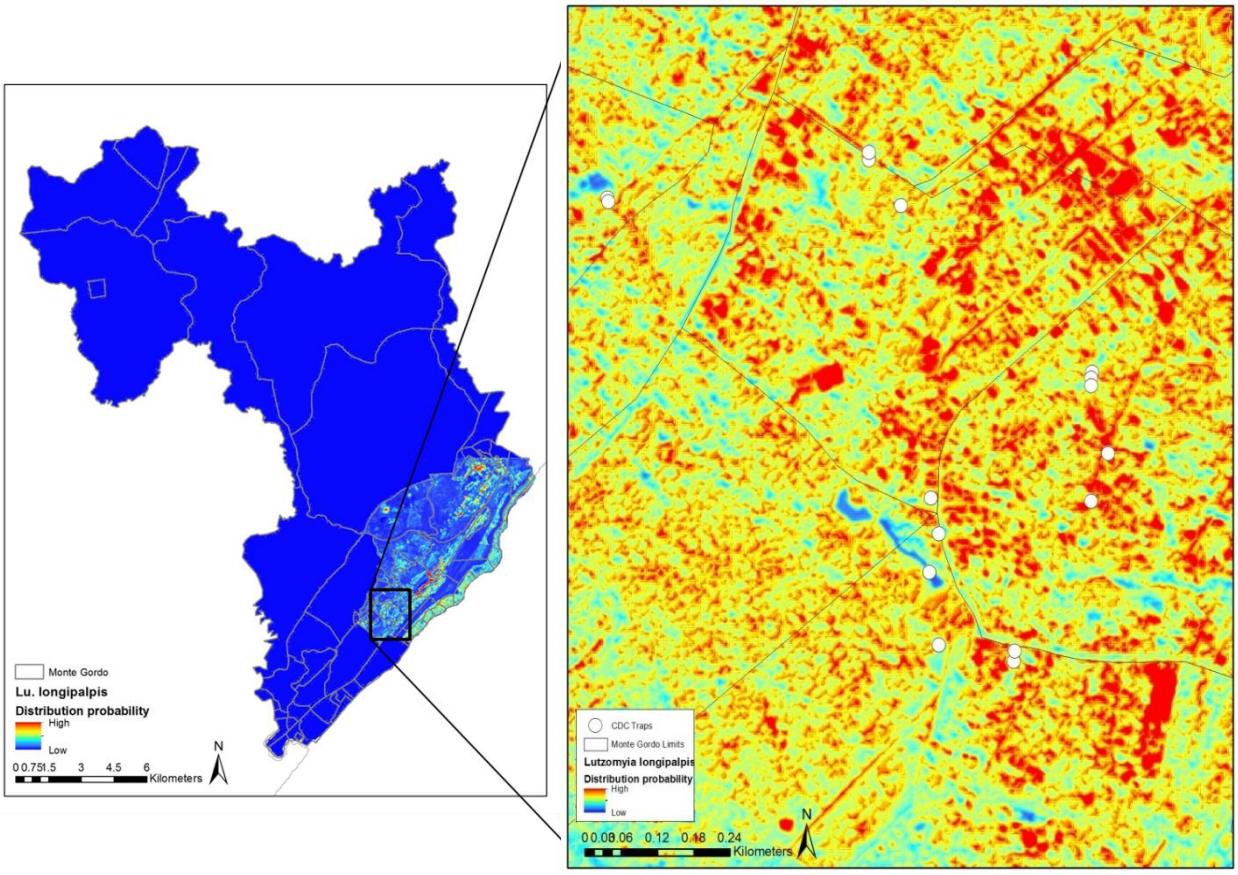

Figure 6.10. Predicted Suitability of *Lutzomyia longipalpis* in Monte Gordo Based on CDC Trap Locations and NDVI, NDSI and NDWI - Red color indicates higher suitability, blue color depicts unsuitable areas.

Developing community level models is important because the community level is really where the control action takes place.

Figure 6.11 shows the natural color composite for the WV-2 image of the area and the vegetation indices derived from that image in gray scale. On Figure 6.12 the vegetation indices are presented in a false color map to show how the indices vary spatially throughout the area and the buffer zones used for data extraction.

6.4. Discussion

A dispersion of VL to new areas has been observed in the last 30 years, especially in the periphery of large cities, including coastal areas. However, the movement of people, entering

the sylvatic environment of the vector, promoting changes to that habitat, is one of the main reasons for the changes observed in the geography of disease. This is reflected in the results recorded in Monte Gordo. Such areas were considered as ecologically inadequate for the presence of *Lu. longipalpis* and thus the establishment of disease (Sherlock, 1996). The district of Monte Gordo belongs to the municipality of Camaçari, an area with the highest records of VL in humans and dogs in the metropolitan region of the Salvador, Bahia state (Barboza *et al*., 2006; Secretaria de Vigilância em Saúde, 2008; Souza, 2014).

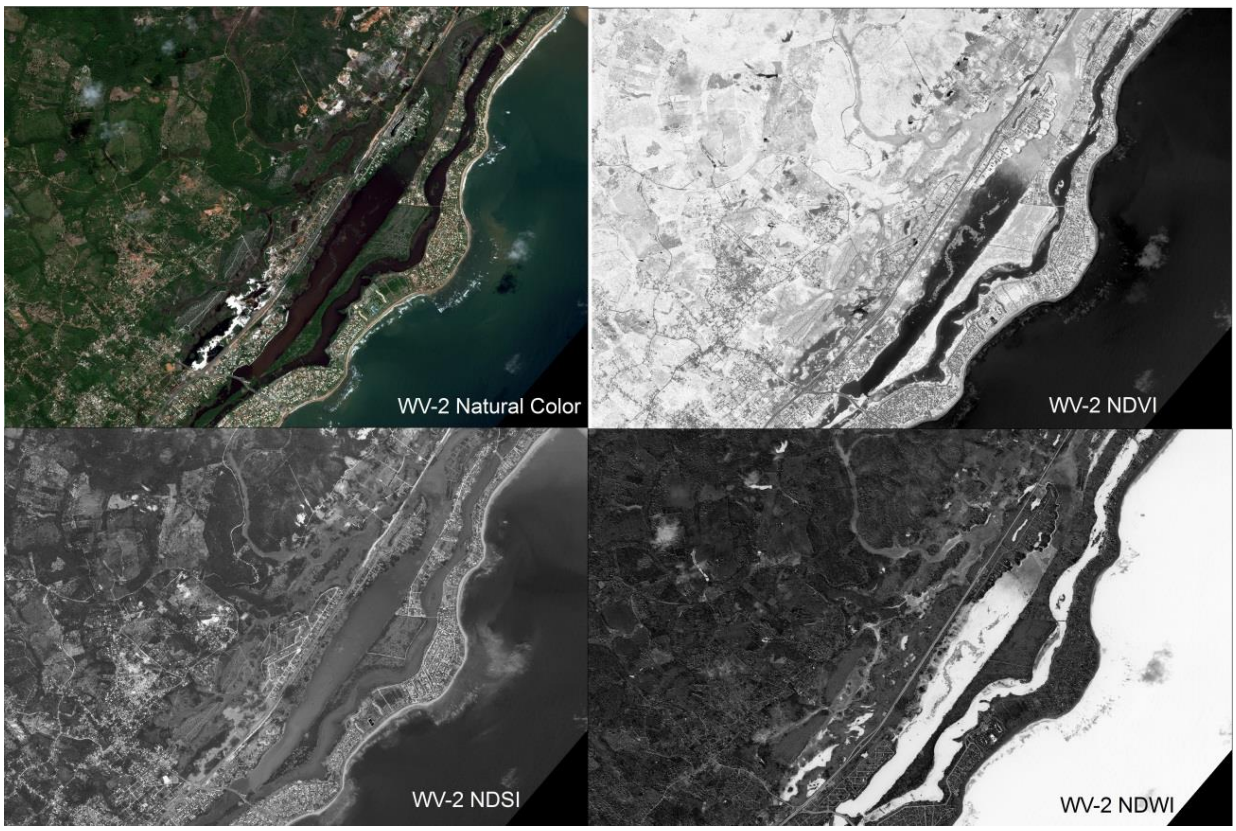

Figure 6.11. WorldView-2 Natural Color Composite and Gray Scale Composite for Vegetation Indices - From top left to bottom right: Natural color composite of WV-2 imagery. Gray scale composite for the vegetation indices: Normalized difference vegetation index (NDVI), Normalized difference soil index (NDSI), and Normalized difference water index (NDWI).

The only sand fly species identified in the present study was *Lutzomyia longipalpis*. The vast predominance of *Lu. longipalpis* in relation to other sand fly species has also been observed in other Brazilian states such as Mato Grosso do Sul and Minas Gerais, in the central

and southeast regions of the country respectively (Almeida et al., 2010; Nunes et al., 2008; Resende et al., 2006). Sousa (2014) and Oliveira et al. (2012), observed an approximate predominance of *Lu. longipalpis* of 95%.

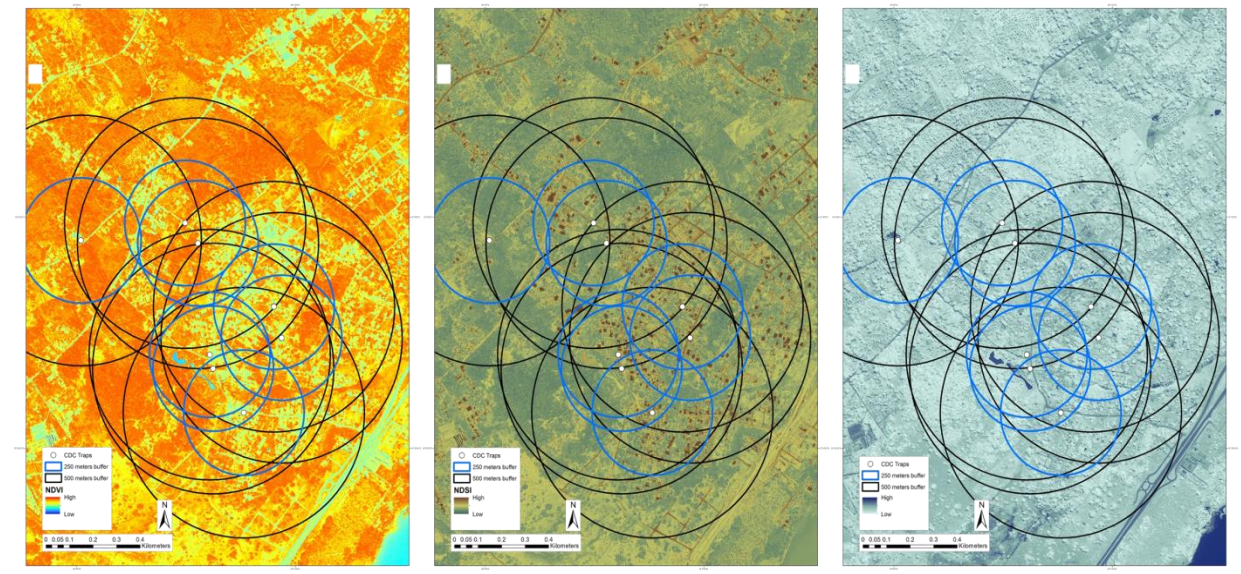

Figure 6.12. RBG Composite of the Vegetation Indices and the Buffer Extraction Zones - From left to right: NDVI: orange areas represent healthy/abundant vegetation; NDSI: green areas represent vegetation while brown areas represent soil material; NDWI: darker blue areas depict water bodies.

A diversity of domestic animals was present in the peridomicile of case study sites. Some authors have reported that in households without domestic animals, sand fly capture is often not successful.

A greater number of male specimens in relation to female sand flies was observed. These results are in agreement with what has been observed in other studies (Almeida et al., 2010; Nunes et al., 2008; Resende et al., 2006). Similar male/female ratios to what was observed in the present study was also found by Oliveira et al. (2012) in Mato Grosso, Brazil. In the municipality of Camaçari, the male/female ratio observed by Sousa (2014) was 3:1. However, Galati et al. (2010), reported a higher proportion of females over males in 21 different species.

The monitoring of sand flies naturally infected with leishmania adds valuable information to understanding vector-borne pathogen transmission cycles (Carvalho et al., 2010; Oliveira-Pereira et al., 2006; Rosa et al., 2012).

Determination of the occurrence of naturally infected sand flies is an important step towards verification of transmission (Killick-Kendrick & Ward 1981). Some authors have reported natural infection rates of leishmania in sand flies between 0.13% to 0.52% (Alves-Pires et al. 2001; Maia et al. 2009; Maia et al., 2013). Valinksy et al. (2014), reported the presence of leishmania DNA in 11% of the samples tested in only one out of three sand fly species tested. In Brazil, studies on natural infection rates in sand flies have shown low infection rates, even in areas of intense VL transmission (Nascimento et al. 2007; Silva et al. 2007; Soares et al. 2010, although a few studies have shown high infection rates of 16.7% and 19% (Lara-Silva et al., 2015; Saraiva et al., 2010).

Natural infection by leishmania parasites was not observed in the engorged females tested in the present study. A previous systematic collection of sand flies over a period of a year in the adjacent area of Camaçari identified a minimum natural infection rate of 1.46%, but infection was not observed throughout the entire year. Only the specimens captured in the months of February, March, May, September and December were infected with leishmania (Sousa, 2014). Feliciangeli (1987), did not observe natural infection of sand flies collected for a period of a year in Venezuela, making it evident that detecting natural infection in some vectors is not always possible, even after continuous sampling of the same area.

The blood meal analysis revealed chickens were the preferred host in Monte Gordo. This association could be due to the fact that chickens are one of the most common domestic animais observed in the area and the attractiveness of sand flies to chickens is often reported (Lainson and Rangel, 2005). Studies done in Ceará and Rio de Janeiro, Brazil captured more sand flies associated with chickens than with humans or dogs (Aguiar et al., 1987; Deane, 1956). Oliveira et al. (2012) and Sousa (2014) observed a difference between sand flies

captured in houses with and without chicken coops. Several studies have evaluated the feeding preferences of sand flies and observed avian species as the preferred food source for *Lu. longipalpis* (Alexander et al., 2002; Dias et al., 2003; De Oliveira et al., 2008; Oliveira et al., 2008). A study in Mato Grosso state showed that *Lu. longipalpis* preferably fed on avian (30.8%) and rodents (21.2%), but human, opossum, cattle, horse and dog blood was also found in their blood meals, as a characteristic of the opportunistic feeding behavior that this vector exhibits (Missawa et al., 2008). The role of chickens in VL epidemiology is unclear. There is no report of avian being reservoirs of leishmania and the existence of infected sand flies in the same area depends on other infected reservoirs such as the dog and fox (Brasil, 2014; Courtney et al., 1995; Otranto et al., 2010).

Even though chickens do not represent a source of infection for sand flies, the presence of this species in the peridomicile attracts the vector. The soil in chicken coops is rich in organic matter that favors the development of immature stages (Afonso et al., 2008). The epidemiological importance of the concentration of sand flies in chicken coops, and their preference for feeding on chickens, is that no vector control is typically applied directly at chickens because these animals are raised for food purposes (Lainson and Rangel, 2005).

The relationship between environmental features and disease agents studied using geotechnologies has provided important information that can be incorporated in surveillance and monitoring programs for several diseases (Bavia et al., 2005; Beck et al., 2000; Malone and Berquist, 2012). The geographic distribution of sand flies is influenced by factors such as precipitation, vegetation, luminosity and abundance of vertebrate hosts (Arias, et al., 1985) and these factors can be mapped and modeled with the aid of geotechnologies such as remote sensing.

The ENM results on sand fly potential distribution in Monte Gordo showed that the geographic distribution of *Lu. longipalpis* is strongly associated with the coastal region of that district. The variable temperature annual range was the most contributing factor in the models.

This variable represents the difference between the highest average summer temperature and the lowest winter average temperature for a given location. Climatic factors are important for developing ENM, due to the long term impact they can have on vegetation and land cover and therefore on the suitability of a habitat for human, animals and insects (Hanafi-Bojd et al., 2015). The population of sand flies is influenced by factors such as temperature, humidity and altitude (Forattini, 1973) and is corroborated by the findings of the ENM for Monte Gordo. A positive correlation between temperature and *Lu. longipalpis* abundance has been reported by some authors (Barata and Apolinario, 2012; Sherlock, 1969). Rivas et al. (2014), observed that temperature affects daily activity of sand flies; under periods of higher temperatures, for example 30°C, L. longipalpis would be not only more abundant, but also more active at night.

Studies on seasonal variation of *Lu. longipalpis* are useful to support programs of integrated vector control, helping to determine the most favorable times of the year to apply insecticides (Resende et al., 2006). These authors observed that the greatest abundance of *Lu. longipalpis* in southeast Brazil corresponded to periods in which the rainfall was highest. Morrinson et al. (1996) observed a significant positive association between female *Lu. longipalpis* abundance and the relative humidity and rainfall three weeks earlier in Colombia, indicating that these factors may be of value in predicting sand fly abundance (Morrison et al., 1996). This species has been reported as being more abundant during or immediately after rainy periods (Souza et al., 2004, Michalsky et al., 2009). Some authors however, have not observed significant correlation between the monthly abundance of sand flies and the monthly averages in rainfall (Costa et al., 2013). Results of the present study regarding precipitation in driest month provides evidence on how rainfall can affect sand flies populations by allowing continuous cycling even at minimum required environmental conditions.

Almeida et al. (2013), in a Maxent approach observed that temperature seasonality and annual precipitation were the variables that most influenced their models for Mato Grosso do Sul state in Brazil. This corroborates the findings of the present study on the influence of

temperature and precipitation on the population of *Lu. longipalpis.* Monte Gordo is located in an area of low thermal seasonality and the results of this study make evident that even slight changes in climatic conditions affect the dynamics of *Lu. longipalpis* in this region.

The occurrence of sand flies has been associated with the presence of vegetation in multiple studies. Different vegetation indices can be used in evaluation of vegetation, and by analyzing the vegetation; one can indirectly evaluate factors such as humidity, temperature and precipitation (Aparicio, 2001; Oliveira et al., 2012). Fernandez et al. (2013), while investigating the spatial and temporal dispersion of *Lu. longipalpis* in Argentina characterized their collection sites mainly by the presence of vegetation, which provides shadow, moist soil, detritus, access to blood sources and a lack of light interference. Soil has been shown to play a significant role in visceral leishmaniasis and is linked to both vector and host (Bhunia et al., 2010). Wasserberg et al. (2002), reported that sand fly density was positively correlated to soil moisture in Israel. Gonzalez et al. (2014), showed in an ENM for *L. longipalpis* in Colombia that soil moisture was the most important factor in building their models. Andrade et al. (2014), using an adjusted soil index in conjunction with two other vegetation indices observed that the central area of Ponta Porã, Brazil presented high values of vegetation, water and soil moisture and also a wider diversity of sand fly species. That area is described as being surrounded by land with remnants of native forest, suggesting a higher degree of moisture in the environment, thus maintaining favorable conditions for vector development. Oliveira et al. (2012) found a significant positive linear correlation between the abundance of *Lu. longipalpis* and the percentage of vegetation cover. Vegetation is important for the maintenance of breeding sites of immature sand fly forms, and it serves not only as a food source for both male and female sand flies, but also provides shelter for vertebrate hosts acting as a food source for the females, thus the importance of understanding the application of vegetation indices in the study of vectors of diseases.

6.5. Conclusion

Analysis of blood meals performed on wild sand flies suggest that such procedures can be applied in vector surveillance programs and provide useful information to be incorporated into control programs by directing actions towards the most preferred host as a way of interrupting the life cycle. The analysis revealed chickens as the preferred feeding source, which was also one of the most common domestic animals observed in the area. The attractiveness of chickens to sandflies is often reported, but their role in VL epidemiology needs to be revisited by the control programs in place.

Natural infection by *Leishmania* was not observed in this study, but monitoring of infection rates in arthropod vectors is essential to investigate the transmission, distribution and spread of disease adds valuable information to understanding vector-borne pathogen transmission cycles.

The community scale study became a proof of concept showing that it is possible to develop ENM at this level, but the models can't be limited to just this scenario because the cycle of disease is much more complicated than just vegetation, climate and presence of vector.

The model developed in this study provides important information to help elucidate the fundamental ecological factors affecting the epidemiology of visceral leishmaniasis in Monte Gordo, Bahia, particularly regarding its vector, *Lu. longipalpis.* Use of WV2 for community based control approaches, at sub-meter pixel resolutions used in this study, make it possible, for the first time, to map and model leishmaniasis and its vectors at habitat and household scales. This may prove to be a crucial approach to the characterization, at community scale, of the multifactorial causation of disease and a way to provide the information essential to correct the currently inadequate control program needed for leishmainaisis.

6.6. References

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Chapter 7: Future Directions

Despite long-standing national policies and programs to control VL and CL, little progress has been made in control since the 1963 government decreed that declared VL and CL to be major public health problems for Brazil. In fact, the situation has worsened in terms of incidence in endemic rural areas and spread to new peri-urban areas not previously affected. This strongly suggests that fresh new approaches and use of novel scientific methods and tools may be needed to make adequate progress in control of VL and CL in Brazil in the future. The focus of dissertaion studies reported here were to develop more effective surveillance and control programs for leishmaniasis using new methodologies made possible by the emergence of geographic information science and molecular epidemiology.

Much data is already being collected as part of current surveillance and control programs, both nationally as a reportable disease by SINAN and in Bahia State by requirements that call for extensive community level investigation of each reported case of VL and CL by LACEN personnel of the Bahia Ministry of Health. Medical history and patient/family interview data are collected, GPS coordinates of the household are recorded and CDC light traps are installed to monitor sand fly populations at monthly intervals for a year. Dogs are tested for serologic evidence of infection, euthanatized if positive, and the presence of other potential animal reservoirs are recorded. LACEN surveillance and investigation data of disease in humans and canines as well as entomological survey data was made available to the investigators for research purposes.

Based on results of studies reported in this dissertation, we propose development and implementation of a comprehensive geospatial health capability for leishmaniasis control in Brazil. ENM probability surfaces were developed to assess risk of leishmaniasis at three scales – at the national scale for Brazil, at the statewide scale for Bahia and, for the first time, at the habitat-household community scale in Monte Gordo, Bahia. Of the three scales, community level models may prove to provide crucial information since the community is where

multifactorial control action takes place and can be evaluated and compared as to efficacy. It is proposed that the elusive success of the control program in place can be improved by implementing a GIS capability where the data that is already being collected can be manipulated and become more epidemiologically meaningful. The rapid identification of areas suitable for transmission by ENM and areas that need immediate intervention may help prevent the occurrence of disease outbreaks and allow efficient allocation of resources for prevention and control at national, state and community scales.

The use of high resolution imagery for mapping at community scale was proven to be possible. The advantage of using high resolution imagery is that no extensive ground survey may be needed to identify suitable habitat and high risk households in areas where access is difficult. However, the use of high resolution images is often limited by cost and technical considerations and the applicability of high resolution images will depend on the size of the area to be studied. High resolution commercial satellite products are available, including QuickBird (2.62 m), Geoeye-1 (1.65 m) and WorldView 2 (1.85 m). A possible operational system for Bahia is to use multispectral Landsat imagery (15, 30 and 60m spatial resolution data) for statewide ENM development and limit use of very high resolution ENM data to reported cases, with randomly chosen control villages to elucidate critical environmental and socioeconomic risk factors. Open source GIS and ENM software are currently available for download at no cost for the user. Grants for acquisition of such data are also available so the cost constraint can be minimized for research applications. Surveillance data of disease in humans and canines as well as entomological survey data can be made available for use in current control programs and for research use.

Additional steps relating to blood meal and natural *Leishmania* spp infection analysis are recommended for inclusion in the surveillance program based on molecular epidemiology research reported here. These data add valuable information to understanding vector-borne pathogen transmission cycles. Further investigations are needed on monitoring of vectors of

disease transmitting agents to make it possible to manage reservoir host populations as a component of future integrated control programs.

Appendices

USDA permits to bring sand flies to the USA.

Failure to supply all applicable information can delay the processing of this application. **PLEASE TYPE OR PRINT CLEARLY**

No controlled material, organisms or vectors may be imported or moved interstate unless the data requested on this form is furnished and certified (9 CFR 94, 95, and 122).

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The time required to complete this information collection is estimated to average .0166 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The valid OMB control number for this information collection is 0579-0015, 0094, 0183, 0213, and 0245.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
National Center for Import-Export, Products Program
4700 River Road, Unit 40
Riverdale, MD 20737-1231

APPLICATION FOR PERMIT TO:

IMPORT OR TRANSPORT CONTROLLED MATERIAL OR ORGANISMS OR VECTORS

1. MODE OF TRANSPORTATION *(Please "X"):*

☐ AIR ☐ SEA ☐ LAND ☒ ANY

2. U.S. PORTS OF ENTRY

As Applicable

3. IMPORTER *(Name, organization, complete address, telephone and fax number of individual who will receive and be responsible for the imported material)*

Dr John B Malone, Pathobiological Sciences, 3203 Veterinary Medicine Bldg, Skip Bertman Dr, Louisiana State Univesity, Baton Rouge. LA 70803, 225 578 9692/9671

4. SHIPPER(s): *(Name and Address of producer/shipper)*

Various Shippers outside the UNITED STATES

5. DESCRIBE THE MATERIAL TO BE IMPORTED *(Provide the following information, as applicable: Animal species and tissue of origin of animal product, country of origin of the animals from which the raw animal product was sourced, processing country, recombinant system and genetic inserts, antibody immunogens, stabilizers, nutritive factors of animal origin in media.) (COMPLETE VS FORM 16-7 for cell cultures and their products.)*

Dead phlebotomine sand flies (Diptera: Psychodidae). All life cycle stages. Sand flies will be stored frozen or in ethanol in 2ml vials.

6. QUANTITY, FREQUENCY OF IMPORTATION, AND EXPECTED COMPLETION DATE *(estimate)*

As many as 10,000 dead sand flies may be transported at a time when collected in field studies. Expected completion date 1Oct15.

7. PROPOSED USE OF MATERIAL AND DERIVATIVES *(Also, for animal pathogens or vectors, describe facilities/biosafety procedures)*

Sandflies will be collected outside the USA for the graduate research program of Moara Martins, supervised by Dr John B Malone of LSU and Dr Lane Foil (LSU Department of Entomology, 412 Life Sciences Bldg). Sand flies will be held in a BL2/ACL2 laboratory (inspected by USDA) - A520 Life Sciences Bldg, LSU, Baton Rouge, LA, USA. Sand flies are imported for identification of species and blood meal

8. IF FOR USE IN ANIMALS, **SPECIFY** THE ANIMAL SPECIES

Not Applicable

9. TREATMENT OF MATERIAL **PRIOR** TO IMPORTATION INTO THE U.S. *(Processing/purification methods, including time at specific temperatures, pH, other treatments, disease safeguards, etc.)*

Specimens will be killed where collected, and will be stored frozen or in alcohol for shipping.

10. METHOD OF FINAL DISPOSITION OF IMPORTED MATERIAL AND DERIVATIVES

Specimens will be slide mounted for ID or ground for PCR blood meal analysis. Unused sand flies will be incinerated.

I CERTIFY AS AUTHORIZED BY THE COMPANY/INSTITUTION THAT I REPRESENT, THAT THIS MATERIAL WILL BE USED IN ACCORDANCE WITH ALL RESTRICTIONS AND PRECAUTIONS AS MAY BE SPECIFIED IN THE PERMIT.

11. SIGNATURE OF APPLICANT

John B Malone

12. TYPED NAME AND TITLE

John B Malone DVM, PhD, Professor

13. DATE

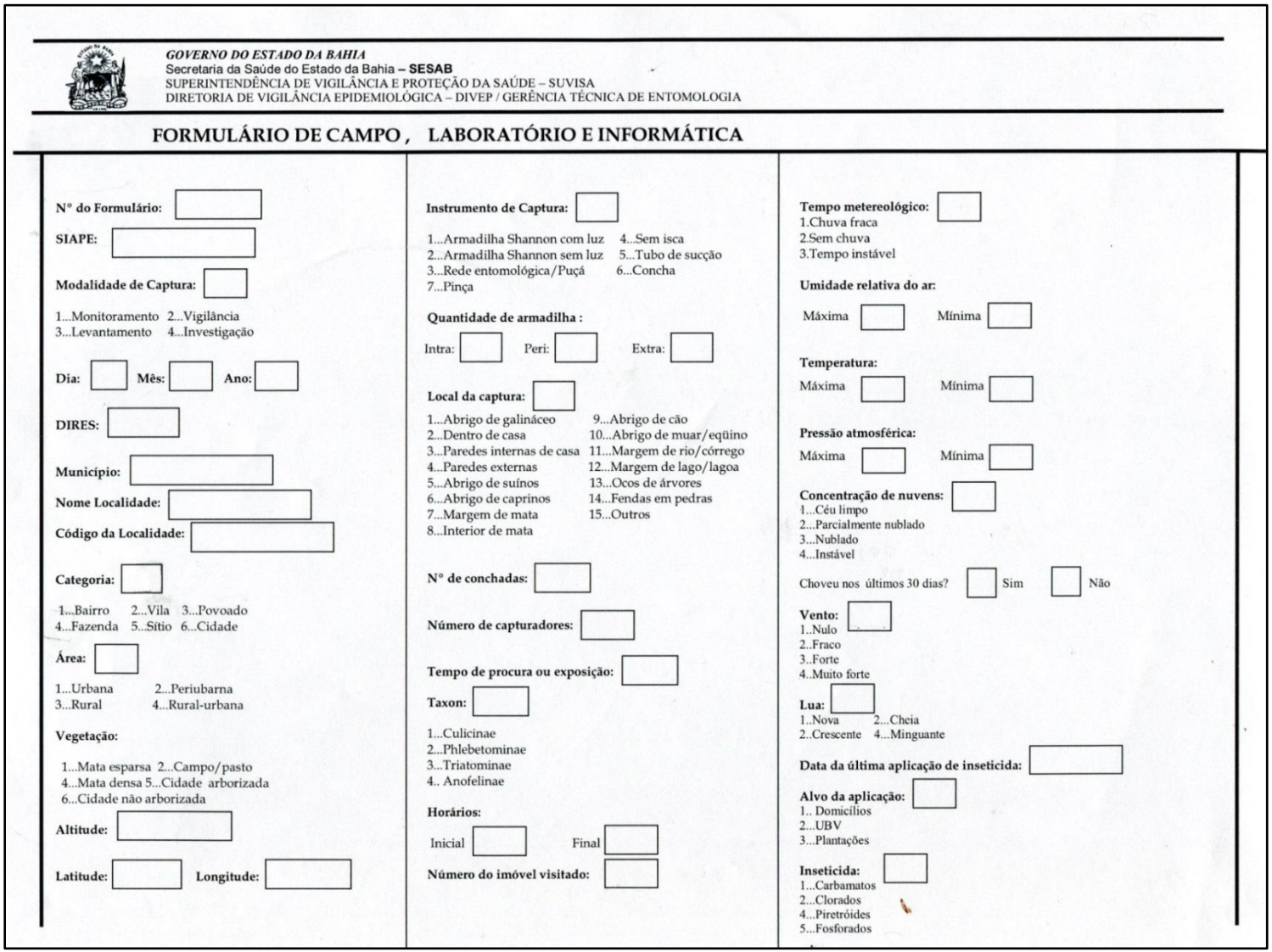
07/10/2015

14. APHIS USER FEE CREDIT ACCOUNT NO. OR METHOD OF USER FEE PAYMENT *(for VISA or Mastercard include number and expiration date).*

VS FORM 16-3 (NOV 99)

Print

Field form filled out by LACEN personnel when placing insect traps.



GOVERNO DO ESTADO DA BAHIA
Secretaria da Saúde do Estado da Bahia – **SESAB**
SUPERINTENDÊNCIA DE VIGILÂNCIA E PROTEÇÃO DA SAÚDE – SUVISA
DIRETORIA DE VIGILÂNCIA EPIDEMIOLÓGICA – DIVEP / GERÊNCIA TÉCNICA DE ENTOMOLOGIA

FORMULÁRIO DE CAMPO , LABORATÓRIO E INFORMÁTICA

N° do Formulário:

SIAPE:

Modalidade de Captura:

1...Monitoramento 2...Vigilância
3...Levantamento 4...Investigação

Dia: **Mês:** **Ano:**

DIRES:

Município:

Nome Localidade:

Código da Localidade:

Categoria:

1...Bairro 2...Vila 3...Povoado
4...Fazenda 5...Sítio 6...Cidade

Área:

1...Urbana 2...Periubarna
3...Rural 4...Rural-urbana

Vegetação:

1...Mata esparsa 2...Campo/pasto
4...Mata densa 5...Cidade arborizada
6...Cidade não arborizada

Altitude:

Latitude: **Longitude:**

Instrumento de Captura:

1...Armadilha Shannon com luz 4...Sem isca
2...Armadilha Shannon sem luz 5...Tubo de sucção
3...Rede entomológica/Puçá 6...Concha
7...Pinça

Quantidade de armadilha :

Intra: Peri: Extra:

Local da captura:

1...Abrigo de galináceo 9...Abrigo de cão
2...Dentro de casa 10...Abrigo de muar/eqüino
3...Paredes internas de casa 11...Margem de rio/córrego
4...Paredes externas 12...Margem de lago/lagoa
5...Abrigo de suínos 13...Ocos de árvores
6...Abrigo de caprinos 14...Fendas em pedras
7...Margem de mata 15...Outros
8...Interior de mata

N° de conchadas:

Número de capturadores:

Tempo de procura ou exposição:

Taxon:

1...Culicinae
2...Phlebetominae
3...Triatominae
4.. Anofelinae

Horários:

Inicial Final

Número do imóvel visitado:

Tempo metereológico:
1.Chuva fraca
2.Sem chuva
3.Tempo instável

Umidade relativa do ar:

Máxima Mínima

Temperatura:

Máxima Mínima

Pressão atmosférica:

Máxima Mínima

Concentração de nuvens:
1...Céu limpo
2...Parcialmente nublado
3...Nublado
4...Instável

Choveu nos últimos 30 dias? Sim Não

Vento:
1..Nulo
2..Fraco
3..Forte
4..Muito forte

Lua:
1..Nova 2...Cheia
2..Crescente 4...Minguante

Data da última aplicação de inseticida:

Alvo da aplicação:
1.. Domicílios
2...UBV
3...Plantações

Inseticida:
1...Carbamatos
2...Clorados
4...Piretróides
5...Fosforados

Vita

Moara de Santana Martins was born in Salvador, Bahia, Brazil. She graduated from Federal University of Bahia, in the city of Salvador, Bahia in 2006, where she earned her Doctor of Veterinary Medicine Degree. While still in the School of Veterinary Medicine, she was awarded a Scientific Initiation Scholarship from the National Council of Research in Brazil. She also received an award from the Brazilian Ministry of Education (CAPES) to participate in an exchange student program which led her to Louisiana State University (LSU). In 2008, she began her work towards a master's degree in Animal Sciences at LSU. In 2010, she worked in a project funded by the Pan American Health Organization (PAHO). Ms. Martins was awarded an internship with the National Aeronautics and Space Administration (NASA) Develop program as a science application consultant and team leader at the Mobile County Health Department in Alabama for two summer semesters. After graduation, Ms. Martins is interested in pursuing her research interests in the field of parasitology associated with environmental health and medical geography.